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LOCAL ANESTHETICS

AN ELECTROPHYSIOLOGICAL INVESTIGATION OF LOCAL ANESTHESIA OF PERIPHERAL NERVES, WITH SPECIAL REFERENCE TO XYLOCAINE

by

JØRGEN RUD

With appendices on

DIFFUSION WITH SIMULTANEOUS INACTIVATION

and

RESTITUTION OF THE NERVE AFTER ANESTHESIA IN TERMS OF DIFFUSION THEORY

by

P. ROSENFALCK, M. Sc.

TRANSLATED

by

M. LENNOX, M. D.

COPENHAGEN 1961

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Denne afhandling er af Københavns Tandlægehøjskole antaget til offentlig at forsvares for den odontologiske doktorgrad.

København, den 21. juni 1960

P. O. Pedersen
Rektor

C. HAMBURGERS BOGTRYKKERI A/S
KØBENHAVN

PREFACE

This study originated from a discussion with Professor KNUD O. MØLLER in 1948 who suggested to me to apply electrophysiological methods to the study of local anesthesia.

The investigation was carried out in the Institute of Neurophysiology, University of Copenhagen during the years 1949 to 1958.

To the director of the institute, Professor FRITZ BUCHTHAL I should like to express my sincere thanks for giving me the opportunity of doing research, for providing excellent facilities and for constant encouragement and support.

I am indebted to Mr. POUL ROSENFALCK M.Sc., the author of Appendix 2 and 3 of this paper, for introducing me to diffusion theory and for numerous and most helpful discussions. My thanks are due to Docent OVE STEN-KNUDSEN M.D. for much good advice and constructive criticism in the evaluation of the results and to Docent J.C. SKOU M.D. for valuable advice and discussion. I also wish to thank Mr. CHRISTIAN GULD E.E. who designed the electronic equipment used in this study and patiently guided me as to its application.

Professor PALLE ANDERSEN kindly reviewed the sections concerning the Ringer's and anesthetic solutions and Mr. OLE SVENSMARK M.Sc. taught me many details of physicochemical technique, Professor K.G. WINGSTRAND performed and evaluated the histological part of the fiber distribution and degeneration experiments and Docent ERNA CHRISTENSEN M.D. made histological sections of a nerve. Mr. VAGN ANDERSEN constructed the experimental chamber. Mr. FLEMMING RIIS has prepared most of the figures. To them I offer my sincere thanks.

The work has been supported by grants from: "Fonden til støtte for videnskabelige og praktiske undersøgelser inden for tandlægekunsten", "Rektor, professor A. Budtz-Jørgensen og Hustru's studielegat til fremme af den odontologiske videnskab" and "The Danish State Research Foundation".

JØRGEN RUD

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B:
BH^+:
C:
C_B:
C_e:
C_m:
C_0:
c.p.s.:
D:
K_a:
k_s:
L_I:
L_{I_1}:
L_{I_2}:
L_{II}:
L_{II_1}:
L_{II_2}:
M:
Megoh
m-equ
msec:
m/sec.
pK_a:
r.m.s.
r_0:
$T_{0.01}$:
$T_{0.5}$:

LIST OF SYMBOLS

B: xylocaine or procaine base.

BH^+: xylocaine or procaine acid.

C: concentration.

C_B: concentration of xylocaine or procaine base.

C_e: concentration of the active anesthetic outside the nerve.

C_m: minimum concentration for xylocaine or procaine base.

C_0: hydrochloride concentration of xylocaine or procaine.

c.p.s.: cycles per second.

D: diffusion coefficient or velocity factor.

K_a: thermodynamic ionization constant.

k_s: acid constant.

L_I: preanesthetic recording electrodes.

L_{I_1}: preanesthetic recording electrodes, distal electrode.

L_{I_2}: preanesthetic recording electrodes, proximal electrode.

L_{II}: postanesthetic recording electrodes.

L_{II_1}: postanesthetic recording electrodes, distal electrode.

L_{II_2}: postanesthetic recording electrodes, proximal electrode.

M: molar.

Megohm: 10^6 Ohm.

m-equiv: milliequivalents.

msec: 10^{-3} second.

m/sec.: meter per second.

pK_a: negative logarithm of the thermodynamic ionization constant.

r.m.s.: root mean square.

r_0: nerve radius.

$T_{0.01}$: time from the moment when the nerve is just blocked and the anesthetic is substituted with Ringer's until the amplitude is 1 per cent of the initial value.

$T_{0.5}$: time from the moment when the nerve is just blocked and the anesthetic is substituted with Ringer's until the amplitude has reached 50 per cent of the initial value.

$t_{0.5}$: time from the application of the anesthetic until the amplitude has been reduced to half.

$t_{0.01}$: time from the application of the anesthetic until the amplitude has been reduced to 1 per cent of its initial value.

INTRODUCTION

Local anesthetics may be defined as agents which, when applied locally in concentrations without local or generalized toxic effects, reversibly arrest impulse conduction along the nerve fibers. Anesthetics affect nerve cells in the peripheral and central nervous system, muscles and neuromuscular transmission as well as peripheral nerves.

An essential aspect of the mode of action of local anesthetics is that they do not abolish the potential difference which exists across the membrane of a resting nerve fiber (HÖBER et al. 1939, TASAKI 1953). Local anesthetics even cause a slight hyperpolarization of the membrane (BISHOP 1932, BENNETT and CHINBURG 1946, SHANES 1951a and STRAUB 1956a). This is in contrast to other anesthetics, such as ether for example, which block the nerve by depolarizing the membrane (LORENTE DE NÓ 1947). The local response as well as the propagated action potential disappear after application of local anesthetics (ROSENBLUETH and RAMOS, 1951, CASTILLO and STARK, 1952 and BENNETT et al. 1942). Local anesthetics do not affect the uptake of oxygen in nerves (LARRABEE et al. 1947, BRINK 1951 as opposed to SHERIF 1930). Cocaine diminishes potassium uptake of normal nerves and nerves with inactivated "active transport" of ions to the same degree. The inactivation of active transport was obtained by anoxia and by monoiodo-acetic acid poisoning (SHANES and BERMAN 1956). This indicated that local anesthetics affect the passive transport of ions and not the active removal of sodium from the cell by the sodium pump. These findings as well as the absence of a depolarizing action of local anesthetics have led to the assumption that their blocking action is due to the fact that they prevent the increase in sodium conductance which normally is associated with the activitation of the nerve fiber (WEIDMANN 1955, STRAUB 1956b).

In myelinated nerve fibers local anesthetics act solely on the nodes of Ranvier, the myelin sheath preventing contact with the nerve membrane (KATO 1936, TASAKI 1939 and WOLFGRAM and HARREVELD 1952). There is evidence of a synergism between calcium ions and local anesthetics (FLECKENSTEIN and HARDT 1949). Thus procaine can repolarize a membrane

which is depolarized by a calcium-free solution (STRAUB 1956b) and cocaine can arrest the spontaneous activity brought about by precipitation of calcium (SHANES 1951a). That lack of calcium ions affects nerve conduction by increasing the permeability of the membrane to sodium (STÄMPFLI and NISHIE 1956, HASHIMURA and WRIGHT 1958, MUELLER 1958) is of special interest, considering the assumed ability of local anesthetics to prevent the increase in sodium conductance which accompanies the normal nerve impulse and thus to block it. In contrast to the synergism between calcium ions and local anesthetics there are signs of an antagonism between the effect of local anesthetics and of veratrine (BUCHTHAL and LINDHARD 1942, SHANES 1950, 1951b, STRAUB 1954, 1956c). Thus local anesthetics can restore impulse conduction in a nerve blocked by veratrine (FLECKENSTEIN 1951). Veratrine increases the sodium permeability of the resting membrane (SHANES 1951b, STRAUB 1954) and the antagonism might be explained by the inhibitory action of local anesthetics on sodium permeability.

The investigations of KATO (1936), TASAKI and TAKEUCHI (1942) and TASAKI (1953) have shown that a local anesthetic applied to a single nerve fiber blocks impulse conduction instantaneously when the concentration of the anesthetic is above a certain concentration, the minimum concentration. A whole nerve is blocked by concentrations above the minimum concentration of a local anesthetic after a time interval which becomes shorter with increasing concentration of the anesthetic relative to the minimum concentration (EHRENBERG 1948). This is due in part to the fact that a certain time is required for the substance to diffuse into the nerve. To evaluate the suitability of a local anesthetic it is thus necessary to know its minimum concentration, the diffusion rate into the nerve and the toxic concentration. The usual clinical tests of local anesthetic effect, such as the corneal test and the subcutaneous or intradermal wheal test, do not determine these characteristics accurately because the experimental conditions cannot be held constant and because the anesthetic effect is reported subjectively. Most of the physiological investigations as to the effects of local anesthetics have therefore been undertaken with nerve-muscle preparations, the absence of muscle concentration serving to indicate full anesthesia. This method is entirely satisfactory to determine the blocking time, i.e. the time when all motor nerve fibers to the muscle in question have ceased to conduct an impulse. The method does not allow the course of anesthesia in the nerve to be followed since its correlation with the degree of contraction is not known. Furthermore, only the effect on motor fibers, comprising less than half the fibers in a mixed nerve, is investigated by this method.

Recording changes in the amplitude of the action potential of a whole

nerve during the course of local anesthesia provides a direct measure of the progress of anesthesia. This method was employed by BENNETT et al. (1942), who measured the time to 80 per cent reduction in action potential amplitude.

An investigation of the time course of anesthesia is of interest for the following reasons:

1) The effectiveness of a local anesthetic is determined by its minimum concentration and by the rapidity with which it penetrates the nerve. A simple and sufficiently precise method for determining these two parameters would be valuable. EHRENBERG (1948) attempted to develop such a method. He applied various concentrations of the anesthetic to the nerve of a nerve-muscle preparation and measured the blocking time. It is in principle possible to determine both the minimum concentration and the diffusion coefficient on the basis of the assumption that the substance penetrates the nerve by free diffusion. EHRENBERG (1948) disregarded a number of complicating factors in the experimental conditions in question. However his method does allow an extremely simple determination of two of the quantities which characterize local anesthetics.

An analysis of the extent to which the course of anesthesia may be described in terms of free diffusion is of interest since a satisfactory agreement would allow prediction of the time course in a whole nerve with various concentrations of a local anesthetic, knowing the minimum concentration and the diffusion coefficient. It seemed therefore of interest to investigate in greater detail the time course of anesthesia and of restitution after anesthesia for various outer concentrations of the anesthetic to obtain information as to how closely the events may be described in terms of a simple diffusion process. Such a study of the time course during the whole of anesthesia has been undertaken in the study reported here.

2) Recording the action potential amplitude during anesthesia of nerves, some with and some without sheath, an attempt was made to determine the effect of the nerve sheath as diffusion barrier.

3) In analyzing the time course of anesthesia in a nerve it must be known which portion of the molecule of the anesthetic is the active anesthetic component. Previous investigations of this problem (GROS 1910, TREVAN and BOOCK 1927, GARDNER, SEMB and GRAHAM 1934, and EHRENBERG 1948) are unanimous that it is the uncharged base component of the local anesthetic molecule which is active as anesthetic agent.

However, SKOU (1954) has found from TREVAN and BOOCK's (1927) and his own investigations that the minimum concentration expressed as the concentration of base varies with pH, which could indicate that the concentration of base is not the sole determinant of anesthetic effect. In the in-

vestigation presented here an attempt was made to contribute to this problem by a) investigating the minimum concentration of xylocaine and procaine at various pH levels and b) investigating the effect of varying the pH on the time course of anesthesia and of restitution, the concentration of base being constant, and c) investigating the time course of anesthesia and restitution at various concentrations of base, with the same hydrochloride concentration at different pH.

4) The recording of the action potential offers the advantage that one can investigate the effect of the anesthetic on the nerve in subminimal concentrations. In the present study the effect of subminimal concentrations on conduction velocity was determined.

5) In these investigations xylocaine (lidocaine, leostesin, lignocaine) was used, one of the most commonly employed anesthetic substances, since LÖFGREN first description of it in 1946. For comparison a smaller number of experiments was carried out with procaine (novocaine). The results are discussed in relation to the clinical use of local anesthetics.

Chapter 1

METHOD

Preparation of the nerves.

The nerves used were sciatic nerves of Hungarian frogs, *Rana esculenta*. The nerve was isolated close to the spinal cord and up to a point about 18 mm distal to the division of the peroneal and tibial nerves. Branches were severed about 5 mm from the nerve.

During dissection the nerve was frequently moistened with a Ringer's solution of the same pH as the anesthetic solution to be used in the experiment. After dissection the nerve was immersed for an hour in this Ringer's solution.

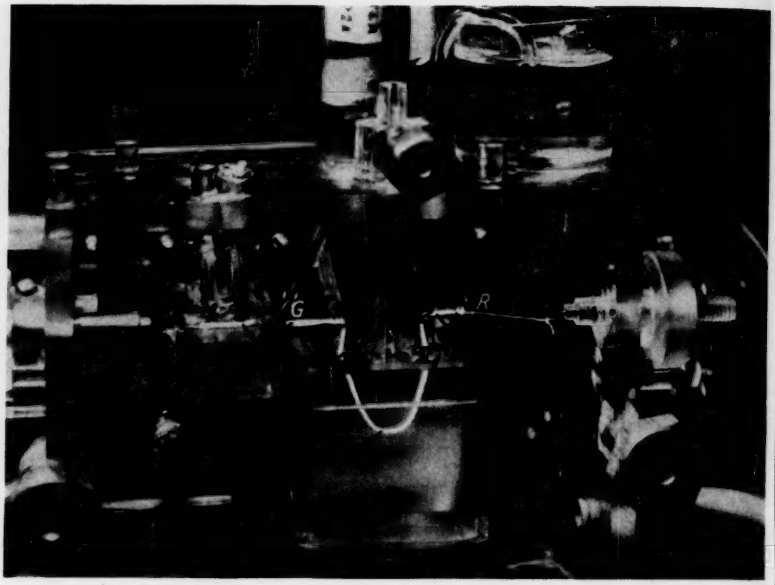
Dissection of the nerve sheath.

A series of experiments was carried out on sheathless nerves. The nerve sheath was dissected away from more than 10 mm of the distal part of that portion of the nerve to be immersed in the anesthetic solution. That the sheath was removed in its entirety was demonstrated by ensuring that it was possible to draw single fibers out of the nerve. That the sheath was entirely removed was also checked by determining the interference colors of the nerve in a polarizing microscope with a red 1st order gipsum plate. Under these conditions the nerve sheath appeared blue with the slow ray of the compensator lying along the fiber axis while the nerve fibers appeared yellow.

Comparing the nerve diameter before and after dissection of the sheath, the thickness of the sheath was found to be 0.04–0.05 mm in agreement with the findings of Lorente de Nó (1950).

Experimental chamber.

The excised nerve was placed in an experimental chamber, where a portion of it could be held submerged in the test solution by means of a perspex pin similar to the procedure used by Larrabee and Posternak (1952) (see Fig. 1).



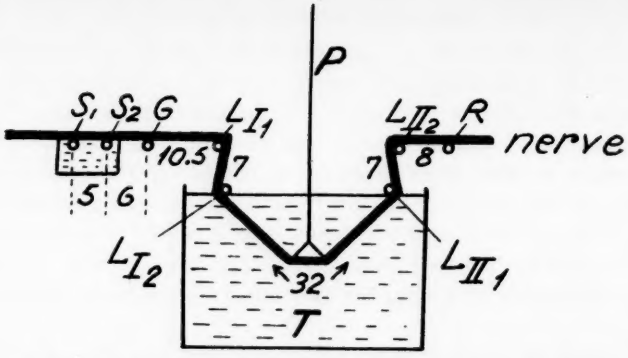


Fig. 1. Experimental chamber and placement of electrodes. N: nerve, S_1 and S_2: stimulating electrodes, G: ground electrode, L_{I_1} and L_{I_2}: pre-anesthetic recording electrodes, L_{II_1} and L_{II_2}: post-anesthetic recording electrodes. P is a perspex rod which holds the nerve submerged in the test vessel T, containing the anesthetic. Electrode R is used in the resistance measurements.

The numbers on the diagram indicate the distances in mm. The nerve is stimulated at its peripheral end.

In this way it was possible to subject a well-defined portion of the nerve to the anesthetic without risking spread of the test solution along the nerve. The nerve was stimulated at its peripheral end (see page 36) and the resulting action potential recorded just before the nerve entered the test vessel and just after it emerged. Thereby it was possible to follow the effect of the anesthetic, both on the amplitude of the action potential and on conduction velocity in the affected section of the nerve lying between the two pairs of recording electrodes. Further details of the experimental chamber are described in Chapter 3 (p. 57).

The experimental chamber was constructed of perspex and designed as a moist chamber. The electrodes consisted of 0.5 mm platinum wires whose bared tips were 5 mm long; except at the tips they were embedded in perspex coated with silicone. Insulation from the moist walls of the chamber was necessary to avoid artefacts. The placement of the electrodes is shown in Figure 1.

To mount the nerve the front wall of the chamber was removed and the test vessel was lowered. The slightly stretched nerve was secured at each end by a cotton thread attached to hooks on the side walls of the chamber. The test vessel was filled with 10 ml of fluid from a syringe and a rubber tube through an opening in its bottom, the fluid surface just touching the lower electrode of each recording electrode pair, L_{I_2} and L_{II_1}. Between electrode L_{I_2} and L_{II_1} the nerve was held down in the test vessel by means of a perspex pin (P). The inner walls of the test vessel were treated with silicone to allow complete removal of all fluid. The walls of the test vessel were double to allow regulation of the temperature by circulating water at the desired temperature. To record the temperature and the pH in the test fluid a thermistor, a glass electrode and a reference electrode could be introduced through the top of the chamber.

Amplifiers.

The action potentials were amplified with an A.C. amplifier with differential input. The amplifier had an input impedance of 100 $M\Omega$ in parallel with an effective capacity to ground of about 5$\mu\mu F$. The amplifier had adjustable maximum and minimum limiting frequency. It was found that distortion of the potential was insignificant when a lower limiting frequency of 1 c.p.s. and an upper limiting frequency of 10^3 c.p.s. were used. The noise level with such a frequency range was less than 2μV r.m.s. and the common mode rejection of the amplifier was more than 10^4. The resulting common mode rejection of the amplifier plus electrodes is given on page 21.

Stimulation.

The rectangular stimulating pulses of 0.15 msec duration were passed through a double screened transformer to isolate the stimulator from the stimulating electrodes. The stimulating electrode closest to the recording electrode was cathode. The stimulus strength was supramaximal for all fibers, in other words two or three times the strength which evoked an action potential of maximum amplitude. The stimulator output was 2–20 volts but the actual stimulating current cannot be given because of the fluid shunt between the stimulating electrodes. The oscilloscope sweep was synchronized from the stimulator. Single pulses could be delivered automatically every other second to record the rapid changes in the action potential.

Artefacts in recording of action potentials.

Since the stimulator and amplifiers were in electrical connection through the nerve, there was a possibility of recording a stimulus artefact superimposed upon and distorting the action potential. In experiments where high amplification was used it was therefore of importance to diminish or eliminate the stimulus artefact. This could be obtained by: 1) isolating the stimulating electrodes from ground. 2) grounding the nerve at a point between stimulation and recording and 3) making use of a high common mode rejection of the amplifier with electrodes.

Concerning 1. The stimulating electrodes were isolated from ground by passing the stimulating current through a transformer. To ensure effective isolation at high frequencies the primary and secondary screens of a General Radio transformer (type 578–A) were connected as described by BUCHTHAL, GULD and ROSENFALCK (1955) (Fig. 2) with one screen connected to the nerve through electrode *C* and the other screen connected to ground.

Concerning 2. The ground electrode *G* was placed on the nerve (Fig. 1) at a distance of more than 6 mm from the nearest recording electrode. Moving the electrode along the nerve it was found that with a given common mode rejection an artefact was recorded when the ground electrode was less than 6 mm from the recording electrode L_{I_1}. The closer the ground electrode was advanced to the recording electrode the greater was the artefact.

Concerning 3. It was important to prevent recording of the potential across the ground electrode common to both recording electrodes. This was ensured by increasing the common mode rejection of the amplifier and electrodes. BUCHTHAL, GULD and ROSENFALCK (1954) have shown that nerve and electrode impedances have a marked effect on the degree of common mode rejection. Since a sufficient common mode rejection was essential

when maximal amplification was used, as at the postanesthetic recording electrodes, the following experiments were performed to determine whether the degree of common mode rejection was adequate:

Both recording electrodes were placed on the nerve. Between the ground and the recording electrodes a 10 mm length of the nerve was substituted by a wool thread wetted with Ringer's solution to interrupt the propagation. With the stimulation intensity and duration usually employed the amplification could be increased to the noise level without recording either action potential or electrotonus, indicating a sufficient degree of common mode rejection.

Recording of the nerve action potential.

The amplified action potentials from the two pairs of electrodes were displayed simultaneously on the two beams of a double beam oscilloscope (Du Mont, type 279).

Before, during and after an experiment a train of signals of known amplitude was supplied from a laboratory generator. Since the deflection of the cathode ray beam was not linear in the periphery of the screen (maximal deviation 6 per cent for amplitude and 13 per cent for time) the potentials were displayed as near the centre of the screen as possible. Furthermore the distance on the screen was compared with the distance between the calibration signals at the same screen position.

The oscilloscope screen was photographed on 35 mm film with a Fairchild camera. Single sweeps were photographed on stationary film to record slow changes. Rapid changes in action potentials were recorded on continuously moving film, the stimuli being delivered once in two seconds. The sweep velocity was 2.7 mm/msec. Though the film moved continuously, the rate was so slow (about 7 mm/sec.) that there was no significant distortion of the record. The time elapsed since the start of the experiment was obtained from the number of recordings. Application of the anesthetic was marked on the continuously moving film.

The action potentials were enlarged 5 to 8 times for measurement.

Measurement of nerve diameter.

The rapidity of the action of local anesthetics on nerve depends on the diameter of the nerve. Measurement of the nerve diameter was carried out through the transparent side walls of the closed chamber. In this way drying of the nerve during measurement was prevented. The nerve diameter was measured immediately proximal to electrode L_1, the thinnest portion of the nerve affected by the anesthetic where blocking first takes place.

The diameter was measured microscopically with 21 times magnification using an ocular micrometer with movable cobweb calibrated against an object micrometer (subdivided into 1/100 mm). The diameter was expressed as the mean of 10 measurements. The mean error of 10 measurements was $\pm$ 0.17 per cent.

Measurement of resistance.

The resistance between the recording electrodes affects the amplitude of the action potential (see p. 41). To determine whether the resistance changed in the course of an experiment and to correct for any such change it was necessary to be able to measure the resistance. Measurement with a bridge arrangement has the disadvantage that the electrode impedances are included; therefore the following procedure was used: the nerve was placed in series with a known resistance. A suitable impulse from a current generator was introduced through this circuit. From the fall in potential between the recording electrodes on the nerve and from the fall in potential over the known resistance, the resistance between the recording electrodes could be calculated (Figure 2):

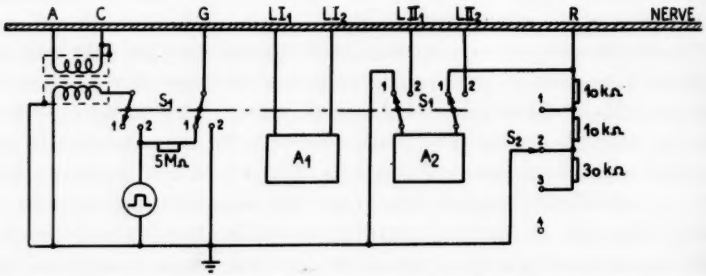


Fig. 2. Arrangement of electrodes for stimulation, recording of action potentials and measurement of resistance between recording electrodes.

For the recording of action potentials switch S_1 is in position 2 and switch S_2 in position 4. The stimulating current passes through the double screened transformer to the nerve between the electrodes A (anode) and C (cathode). Electrode G is grounded and amplifiers A_1 and A_2 are connected with electrodes L_{I_1} and L_{I_2} for the pre-anesthetic recording and with electrodes L_{II_1} and L_{II_2} for the post-anesthetic recording.

For the measurement of resistance S_1 is in position 1 and S_2 in position 1, 2 or 3. With the stimulator as generator a current is passed via electrode G, the nerve, electrode R and a known resistance to ground. Amplifier A_1 records the potential over the portion of nerve between electrodes L_{I_1} and L_{I_2}, where it is desired to measure the resistance, and amplifier A_2 records the potential over the known resistance.

From the potential over the nerve (E_N) and the potential (E_R) over the known resistance (R) the nerve resistance was found as: $R_N = R \cdot E_N/E_R$.

In checking the accuracy of the method with a known resistance in place of the nerve, the mean error of 10 measurements was $\pm$ 0.75 per cent. Measuring the resistance of a nerve in air 10 times at two places, the mean error was $\pm$ 3.8 and $\pm$ 4.3 per cent.

Determination of the pH of the test fluid.

Since the effect of local anesthetics varies with the hydrogen ion concentration of the solution, it was necessary to measure the pH of the solution continuously during the experiments.

pH was measured with a pH meter (Radiometer type P.H.M. 22). The glass electrode (Radiometer G 222 A, diameter: 4.5 mm, resistance: 2–300 $M\Omega$ at 20°C) was introduced directly into the test fluid. The calomel-potassium chloride reference electrode was introduced into a beaker containing saturated potassium chloride and contact with the test fluid was obtained through a glass tube 10.5 cm long and with a 2 mm lumen filled with Ringer-agar (99 per cent Ringer's solution and 1 per cent agar). Because of this "liquid junction" the measured pH was 0.25 units greater than if measured with the reference electrode directly in the test chamber. The deviation was constant over the pH range 4–8.

The pH apparatus including the Ringer-agar junction was adjusted using potassium hydrogenphthalate 0.05 molar (pH 4.005) and sodium borate 0.05 molar (pH 9.18) as buffer solutions.

Humidity and temperature in the experimental chamber.

Humidity: To avoid drying of the nerve the air in the chamber had to be saturated with moisture. This was obtained by bubbling an oxygen-carbon dioxide mixture through four water bottles using special glass filters which gave very small bubbles. When the bottles were placed in a water bath at 40–50°C, a supersaturated moisture was obtained at the temperature of the chamber. The supersaturated air was conducted through insulated tubes to the chamber. Furthermore a sponge wetted with Ringer's solution was placed on the floor of the chamber. To ascertain that full saturation of the air in the chamber was maintained a thermo-couple wrapped in moist cotton was introduced into the chamber. At constant temperature in the chamber no evaporation from the cotton was seen over a period of many hours.

Temperature in the test vessel was measured with a standard thermistor (Standard Electric, type U), which was placed as one resistance in an alternating current bridge. The tip of the thermistor was in contact with the nerve. The temperature could be read with an accuracy better than 0.1°C.

Because of the high temperature of the oxygen-carbon dioxide which was passed through the chamber the temperature of the nerve had a tendency to rise during the experiment. This rise in temperature could be compensated by running cool water through the double wall surrounding the test chamber. Different temperatures were obtained by running water of a suitable temperature through the double wall of the experimental chamber. In these experiments the liquid in the test vessel was stirred by means of a motor-driven propeller.

Measurement of minimum concentration.

To avoid too long an experimental time, Hungarian frogs were used (Rana esculenta) small in size and therefore with thin nerves (about 0.30 mm in radius). Immediately after dissection the nerves were placed for one hour in Ringer's solution at the pH to be used during anesthesia. Thereafter each nerve was mounted singly in the experimental chamber (Figure 1), lying from the site of the stimulating electrodes S_1 to S_2 across the ground electrode to the recording electrodes L_{II_1} and L_{II_2}. In every series of experiments the action potential was recorded for each of 10 nerves in Ringer's solution whereafter the 10 nerves were mounted in such a way that the distal end of each nerve (the site of stimulation) was contained in a Ringer filled tube 20 mm long sealed at each end with vaseline. The approximate 45 mm long proximal section of each nerve was suspended slightly stretched by cotton thread and was immersed in the anesthetic solution.

The longest blocking time measured in 46 experiments with various xylocaine concentrations was two hours (experiment 156, $C_B = 0.15$ mM). It was therefore considered that an experimental time of six to eight hours should be sufficient to attain diffusion equilibrium between the bathing fluid and the interfibrillar fluid of the nerves (see p. 76).

To maintain a constant pH the anesthetic solution was bubbled with oxygen carbon-dioxide mixture (by means of glass filters) during the whole anesthetic period.

After 6–8 hours in the anesthetic solution the nerves were replaced in the experimental chamber and the degree of block in each nerve was determined from the amplitude of the action potentials evoked. The minimum concentration was determined as the smallest concentration at which about half

of the 10 nerves were totally blocked and the majority of the remaining nerves showed a substantial reduction in action potential amplitude.

To ensure that the nerves which were blocked after anesthesia were undamaged, the nerves were transferred again to Ringer's solution. Only those nerves which showed restitution of the action potential were included in the experimental material.

Chapter 2

ANESTHETIC AND RINGER'S SOLUTIONS

Local anesthetics are alkaloids which in the presence of acids, form stable salts easily soluble in water. In this study only xylocaine and procaine hydrochloride were studied. In the concentrations used the salt was completely dissociated into acid and Cl^-. In watery solution the following protolytic equilibrium obtains:

$$BH^+ + H_2O \rightleftarrows B + H_3O^+ \qquad (1)$$

where B denotes the free base which is uncharged and BH^+ denotes the acid. More salt is formed when hydrogen ions are introduced into the solution, while the concentration of the base is increased when the solution is rendered basic. Solutions with different concentrations of the free base could be obtained by changing the salt concentration or by varying the pH of the solution (see p. 29).

To maintain a constant concentration of free base during the experiments it was necessary to maintain a constant pH and buffer solutions were added to ensure a high buffer capacity. One of the physiological buffers is carbon dioxide-sodium bicarbonate which regulates the intracellular hydrogen concentration (Ahlgren 1930). In addition carbon dioxide is thought to have a specific effect on the metabolism of nerve (see p. 48). Three per cent carbon dioxide-bicarbonate buffer was therefore added to the solutions (see p. 31) in addition to a phosphate buffer to obtain a sufficient buffer capacity (see p. 31).

To avoid the effect of other factors than the concentration of the anesthetic and the pH of the solution, all solutions, both Ringer's and anesthetic were adjusted with respect to the osmotic pressure (249 m-equiv) and a constant concentration of Ca^{++}, K^+ and Na^+, while the concentration of Cl^- could be varied.

When the anesthetic was added to Ringer's solution, it was necessary to reduce the total concentration of sodium chloride to maintain isotonicity.

The usual sodium content of the Ringer's solution was therefore decreased from 115 mM to 75 mM. The effect of this reduction in sodium concentration on nerve activity is discussed on p. 50. Up to 40 mM of anesthetic could be added to this solution without altering its isotonicity. The Ringer's solution as well as the anesthetic solutions with concentrations less than 40 mM had to be supplemented with an inactive component until the osmotic pressure corresponded to 249 m-equiv. Glucose might be considered as an inactive component. LORENTE DE NÓ (1947) states, however, that glucose can produce irreversible changes in nerves in the presence of anoxia. Choline chloride was therefore used instead, which according to LORENTE DE NÓ (1947) has no effect on the nerve impulse.

Protolysis of xylocaine and procaine acid.

The protolytic equilibrium (1) gives:

$$\frac{a_B \cdot a_{H_3O^+}}{a_{BH^+}} = \frac{C_B \cdot f_B \cdot a_{H_3O^+}}{C_{BH^+} \cdot f_{BH^+}} = K_a \qquad (2)$$

where K_a is the thermo-dynamic ionization constant, a denotes the activity and C the molar concentrations of the substances involved in the equilibrium. f_B and f_{BH^+} are the activity coefficients.

Since B is uncharged, f_B is assumed equal to 1. In addition

$$C_{BH^+} + C_B = C_0 \qquad (3)$$

C_0 representing the concentration of xylocaine or procaine hydrochloride. (2) may therefore be written:

$$C_B = \frac{C_0}{\frac{a_{H_3O^+}}{K_a \cdot f_{BH^+}} + 1} \qquad (4)$$

From this expression the concentration of free base (C_B) may be derived when the following quantities are known:

1) the concentration of xylocaine- or procaine hydrochloride (C_0)
2) the hydrogen ion activity ($a_{H_3O^+}$)
3) the thermodynamic ionization constant (K_a)
4) the activity coefficient (f_{BH^+}).

Ad. 1. The anesthetic solutions were prepared with a known concentration of xylocaine or procaine hydrochloride.

Ad. 2. The hydrogen ion activity may be determined by measuring the pH of the solution, since the method used for measuring pH gives the following relationship according to DOLE (1941):

$$\mathrm{pH} = -\log a_{H_3O^+} \tag{5}$$

Ad. 3. LÖFGREN (1948) found the thermodynamic ionization constant K_a for xylocaine acid to be $10^{-7.85}$ at 25°C. K_a for procaine acid was calculated from the results of experiments performed by EISENBRAND and PICHER (1938). They measured the pH of various procaine solutions to which sodium hydroxide had been added. Calculating the ionic strength (μ) for these solutions, and using the value $k_s = 10^{-8.95}$ found by EISENBRAND and PICHER (1938), it is found that $K_a = 10^{-8.93}$, using the formula of HÄGG (1948) which is based on the fact that pH measurements reflect the hydrogen ion activity:

$$pk_s = pK_a + 0.5\,(z_s^2 - z_b^2)\,\sqrt{\mu} \tag{6}$$

with z_s and z_b as the valencies for the acid and the base and μ ionic strength.

Ad. 4. The activity coefficient (f_{BH^+}) is calculated with fair approximation, using the DEBYE-HÜCKEL equation:

$$-\log f = \frac{0.5\,z^2\sqrt{\mu}}{1+\sqrt{\mu}} \tag{7}$$

with μ representing the ionic strength of the whole solution.

μ may be calculated from

$$\mu = 0.5\,\Sigma C z^2 \tag{8}$$

when C is the concentration of the single ions in solution and z their valency.

Frog Ringer's solution contains the following salts: NaCl: 115 mM, KCl: 2.7 mM, $CaCl_2$: 1.8 mM and $NaHCO_3$: 3.7 mM. Substituting some of the sodium chloride in the Ringer's solution with other salts containing 2 ions with valency 1, there is no change in ionic strength as long as the total concentration is the same. This is the case for choline chloride, approximately so for sodium bicarbonate as well as for xylocaine and procaine hydrochloride. From the dissociation of the salt:

$$BH^+ + Cl^- + H_2O \rightleftarrows B + H_3O^+ + Cl^- \tag{9}$$

it is seen that it makes no differences how the equilibrium is shifted; there will only be two charged ions with one charge each. In substituting phos-

phate buffer, the ionic concentration is increased. With the phosphate buffer:

$$NaH_2PO_4 + NaOH = Na_2HPO_4 + H_2O \qquad (10)$$

with a concentration of NaH_2PO_4 of 0.0066 M, only NaH_2PO_4, is present at low pH (ionic strength of 0.0066). At high pH only Na_2HPO_4 is present with an ionic strength of 0.0198, in other words an *increase* in ionic strength of the Ringer's solution of 0.0132. At a mean value of pH (6.82) the increase in ionic strength from the phosphate buffer is 0.0066. This added to the ionic strength of 0.127 for Ringer's solution results in a total ionic strength of 0.134. Substituting this ionic strength in the DEBYE-HÜCKEL equation ((7) p. 28) the activity coefficient $f_{BH^+} = 0.73$.

In the experiments C_0 values were used of 1, 2, 5, 20 and 40 mM. The corresponding values for C_B at various pH levels may be calculated from equation (4) (Fig. 3).

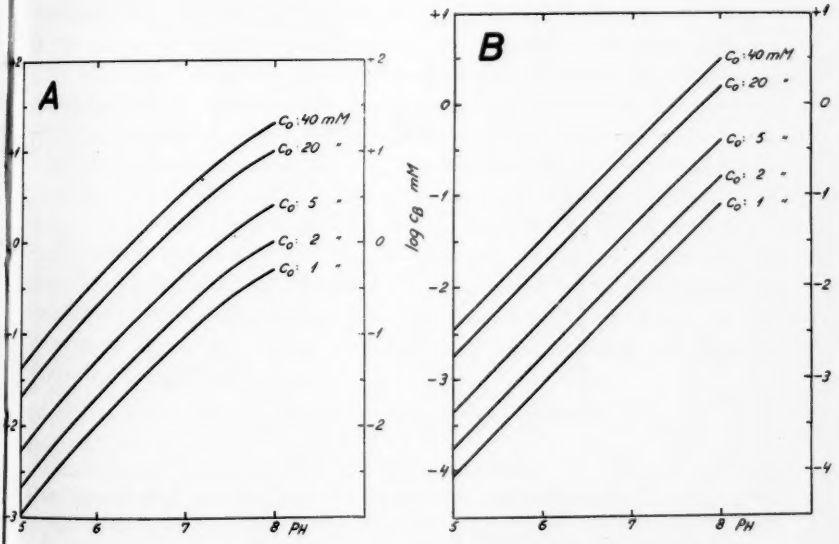


Fig. 3. Protolysis of (*A*) xylocaine and (*B*) procaine acid at various pH values.
Ordinate: logarithm of the base concentration in mM.
Abscissa: pH.

The curves represent various initial concentrations of xylocaine and of procaine hydrochloride (C_0) in mM at 25°C.

Carbon dioxide-bicarbonate buffer.

The amount of bicarbonate and carbon dioxide which must be added to the solution to obtain a certain pH can be calculated from the HENDERSON-HASSELBALCH equation:

$$\mathrm{pH} = pk' + \log \frac{C_{\mathrm{HCO_3^-}}}{C_{\mathrm{CO_2}}} \tag{11}$$

with pk' as the first ionization constant of the carbonic acid in water. According to HARNED and DAVIS (1943) this is 6.326 at 30° C. The pk' increases by 0.005 per degree fall in temperature. CO_2 and HCO_3^- concentrations in the equation are expressed in M.

The carbon dioxide pressure can be expressed in M by the following expression:

$$\mathrm{CO_2}\,(M) = \frac{P \cdot \alpha \cdot n \cdot 1000}{760 \cdot 22.400 \cdot 100} \tag{12}$$

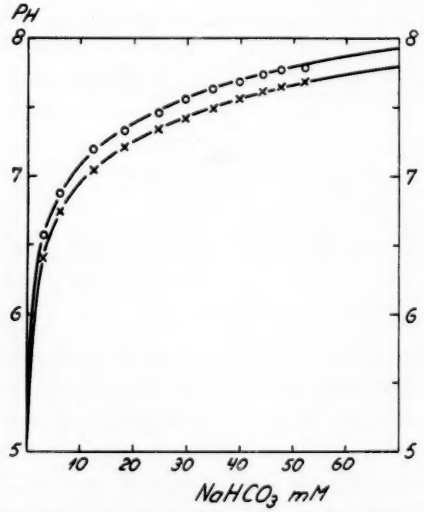


Fig. 4. pH of the carbon dioxide – bicarbonate buffer as a function of sodium bicarbonate concentration when the solution is saturated with 5 per cent CO_2.
○: experimental values in distilled water;
×: experimental values for Ringer's solution.
The curves were calculated from the Henderson-Hasselbalch equation for a watery solution and for a solution with the ionic strength of Ringer's.
Ordinate: pH.
Abscissa: concentrations of $NaHCO_3$ in mM.

when P is atmospheric pressure in mm Hg, n is the per cent content of CO_2 in the air mixture. α is the HENRY's law constant for carbon dioxide in water (0.87 at 20°C, HARNED and DAVIS 1943).

Figure 4 shows how much bicarbonate had to be added to 1 litre of water saturated with 5 per cent carbon dioxide at 760 mm Hg and at 20°C to obtain pH values between 5 and 7.8. Experiments with distilled water and buffer showed agreement with the values calculated from the HENDERSON-HASSELBALCH equation.

In experiments with Ringer's solution it was found that 40–46 per cent more bicarbonate must be added to obtain the same pH. The reason for this is the effect of the ionic strength of the Ringer's salts. To compensate the theoretical values with respect to ionic strength the dissociation constant k_s must be used instead of the thermodynamic constant K_a.

k_s may becalculated from the following expression:

$$pk_s = pK_a + \frac{0.5\,(z_s^2 - z_b^2)\sqrt{\mu}}{1 + \sqrt{\mu}} \qquad (13)$$

where z_s and z_b are the valencies of acid and base and μ is the ionic strength of the solution. Since $z_s = 0$ and $z_b = -1$, μ is 0.134 and pK_a at 30°C is 6.33, pk_s is 6.20 at 30°C. At 20°C pk_s is 6.25. Calculating the amounts of bicarbonate with this dissociation constant a relationship was obtained which corresponded closely to the experimental data (Figure 4).

At pH 7 it was necessary to add 11 mM/l bicarbonate corresponding to 22 m-equiv. At higher pH the amount to be added increased sharply. Thus at pH 7.7 110 m-equiv bicarbonate had to be added or 44 per cent of the total osmotic pressure of the Ringer's solution. When pH exceeded 7, so much bicarbonate had to be added that the solubility product of calcium carbonate was exceeded and calcium ions were thrown out of the solution. The use of 3 per cent carbon dioxide instead of 5 per cent reduced these difficulties considerably.

Phosphate buffer.

EHRENBERG (1948) indicates that phosphate buffer up to 10 mM does not alter the sensitivity of nerves to local anesthetics. Phosphate buffer in higher concentrations causes the nerves to be anesthetized more readily. This is perhaps due to the fact that free calcium ions, which are necessary to maintain nerve stability, are bound as insoluble calcium phosphate.

Phosphate buffer is formed as:

$$NaOH + NaH_2PO_4 \rightleftarrows Na_2HPO_4 + H_2O \qquad (14)$$

The concentration of NaH_2PO_4 used was 6.6 mM (LORENTE DE NÓ 1947). To calculate the amount of sodium hydroxide to be added to obtain a desired pH in phosphate-buffered Ringer's solution the dissociation constant k_s at the ionic strength of the solution had to be known. Since the solution in question was relatively concentrated, the dissociation constant could be calculated from the expression of COHN (1927):

$$pk_s = pK_a - \frac{1.5\sqrt{\mu}}{1 + 1.65\sqrt{\mu}} \tag{15}$$

Cohn (1927) determined pK_a to be 7.16. Since the ionic strength is 0.134, $pk_s = 6.82$.

With various amounts of sodium hydroxide the pH could be calculated from:

$$\text{pH} = 6.82 + \log \frac{C_{\text{NaOH}}}{C_{\text{NaH}_2\text{PO}_4} - C_{\text{NaOH}}} \tag{16}$$

where the concentration of sodium hydroxide (C_{NaOH}) equals the concentration of Na_2HPO_4 ($C_{NaH_2PO_4}$).

Figure 5 shows the agreement between the theoretical curve and the experimental values.

Sodium hydroxide added to xylocaine or procaine Ringer's solution combined partly with NaH_2PO_4 and partly with xylocaine or procaine hydrochloride as follows:

$$\text{NaOH} + \text{BHCl} \rightleftarrows \text{NaCl} + \text{H}_2\text{O} + \text{B} \tag{17}$$

where B is the uncharged xylocaine or procaine base; i.e. the amount of B formed was equal to the amount of sodium hydroxide bound. The value of B at various pH values was calculated from equation (4) (see the curves in Fig. 3). At the same time the amount of sodium hydroxide bound by the anesthetic was obtained. The total amount of sodium hydroxide required to obtain a certain pH in a phosphate buffered xylocaine or procaine Ringer's solution of a given concentration of hydrochloride could then be calculated.

Preparation of Ringer's and anesthetic solutions of different concentrations and pH.

It was requisite that all solutions, Ringer's as well as anesthetic solutions, be isotonic and with an osmotic pressure corresponding to 249 m-equiv. In addition, all solutions had to contain concentrations of potassium, calcium

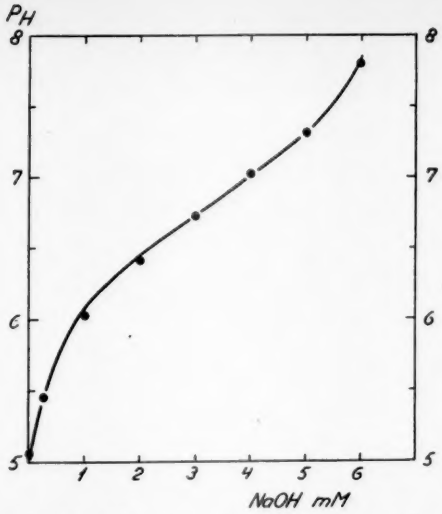


Fig. 5. pH of the phosphate buffer in Ringer's solution as a function of the concentration of NaOH for a solution containing 6.6 mM NaH_2PO_4 and with an ionic strength of 0.134.
●: experimental values with phosphate buffer in Ringer's solution.
The solid line is calculated with a pk_2 of 6.82.
Ordinate: pH.
Abscissa: concentration of NaOH in mM.

and glucose as in normal frog Ringer's solution, as well as the reduced sodium concentration of 75 mM.

In order to fulfill these demands and to be able to vary the concentration of anesthetic between 0 and 40 mM and to vary the pH of the buffered solutions between 5.5 and 7.8, 6 different stock solutions were prepared and mixed in suitable proportions to give a solution with desired properties.

In appendix 1 the stock solutions are described in detail and the proportions in which they were mixed to obtain given Ringer's or anesthetic solutions.

Chapter 3

MEASUREMENT OF THE AMPLITUDE OF THE ACTION POTENTIAL AND OF THE CONDUCTION VELOCITY OF THE NERVE IMPULSE

CONDITIONS INFLUENCING THE NERVE ACTION POTENTIAL AND ITS CONDUCTION VELOCITY

In this chapter an account is given of the factors which affect action potential amplitude and conduction velocity. Some of these are well known from the literature (temperature, carbon dioxide tension, pH). Others have not previously been investigated in detail, such as the distance and the resistance between the recording electrodes and the degree of stretch of nerve. With regard to the influence of temperature, carbon dioxide tension and pH only few experiments were carried out to ensure that the experimental arrangement allowed the results reported by others to be reproduced. The influence of distance and resistance between the recording electrodes and stretch of the nerve were studied in greater detail.

The stimuli applied to the nerve had a duration of 0.15 msec. and a strength 2–3 times the strength which evoked an action potential of maximum amplitude. According to Gasser and Erlanger (1937) such a stimulus activates α, β and γ fibers of the A group but not B and C fibers. According to Gasser and Erlanger (1937) the threshold of B fibers is 53.3 and of C fibers is 100 times the threshold of α fibers. With the time scale used the action potentials of B and C fibers would not appear on the oscilloscope trace.

The nerve impulse was led off with two electrodes on the nerve, each recording a monophasic potential. Since the distance between the recording electrodes was less than the segment occupied by the monophasic potential, the difference between the two monophasic potentials was recorded as a diphasic potential.

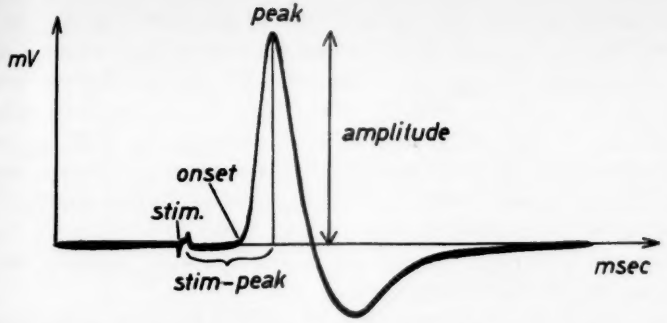


Fig. 6. The definition of action potential amplitude and of measuring points for the determination of conduction velocity.

Definitions.

Action potential amplitude.

The action potential amplitude was measured in mV from the base line to the maximal negative deflection (Fig. 6). The amplitude measured on ten successive action potentials recorded from the same nerve showed a mean error of $\pm$ 0.2 per cent. The mean amplitude measured proximally to the test vessel on 63 different unanesthetized nerves was 5.88 mV $\pm$ 3.8 per cent.

With a noise level of 2μV r.m.s. the smallest action potential which could be measured was 10–30 μV in amplitude, i.e. 0.2–0.5 per cent of the amplitude before anesthesia. To estimate how many nerve fibers contribute to this potential the number of nerve fibers was counted in a cross section of the nerve near the stimulating electrodes (Table 14). About 1300 large fibers with myelin sheath were found. Assuming that each fiber contributes equally to the total action potential, an action potential amounting to 0.2–0.5 per cent of the initial amplitude arises from 3–6 nerve fibers. That there were only few nerve fibers active when the smallest recorded action potential was obtained is also appearent from experiments on single nerve fibers (Adrian and Bronk 1928, Hertz 1947). With leading-off conditions similar to those in the present experiments an amplitude of approximately 20–50 μV was found.

Action potential conduction velocity.

The conduction velocity was calculated in m/sec from the time of impulse conduction between the two sets of recording electrodes and the distance between them. The point on the action potential used for the determination

of conduction velocity was the peak of the potential (Fig. 6). The time interval between the peaks of the pre- and postanesthetically recorded potentials represented the conduction time over the section of nerve between electrodes L_{I_1} and L_{II_1} (Fig. 1). There were 7 mm between electrodes L_{I_1} and L_{I_2} in air and 32 mm between electrodes L_{I_2} and L_{II_1} in the anesthetic solution. Since the main object was to measure conduction velocity over the anesthetized section of nerve, the total conduction time had to be corrected for the conduction time over the 7 mm of nerve in air. To this purpose the conduction velocity was determined with the whole nerve in air before the anesthetic experiment.

The conduction velocity measured 10 times in the same nerve showed a mean error of $\pm$ 0.2 per cent. The mean conduction velocity in Ringer's solution at 22°C for 10 different nerves was 31.3 m/sec. $\pm$ 2.1 per cent.

Amplitude and conduction velocity of the action potential measured on a whole nerve in relation to measurements on single fibers.

The action potential recorded from a whole nerve is the sum of the action potentials of the single fibers. Since these conduct at various velocities there results a temporal dispersion along the nerve. GASSER and ERLANGER (1937) analyzed the shape of the action potential of whole nerve as a function of the distance from the stimulating point and interpreted the shape in terms of the distribution of fiber diameters, conduction velocities and spike amplitudes.

Amplitude of the action potential.

Under the conditions of the experiments reported here temporal dispersion and differences in the external shunt resulted in a higher amplitude of the action potential measured at L_I (preanesthetic recording) than recorded at L_{II} (postanesthetic recording). The amplitude recorded at L_I was higher when the nerve was stimulated proximally than when it was stimulated distally. This was due to the fact that fibers branch off*) from the main nerve between the two recording points. When the stimulating point was proximal the action potentials of these fibers were recorded at L_I but not at L_{II}. When the stimulation was distal the same number of fibers was recorded from both points since all stimulated fibers extended throughout the nerve. For this reason the nerve was always stimulated distally in the experiments concerning anesthesia.

*) Ramus profundus auterior and posterior leave the sciatic nerve 16 mm proximal to electrode L_{I_2} when the nerve is stimulated at the distal end.

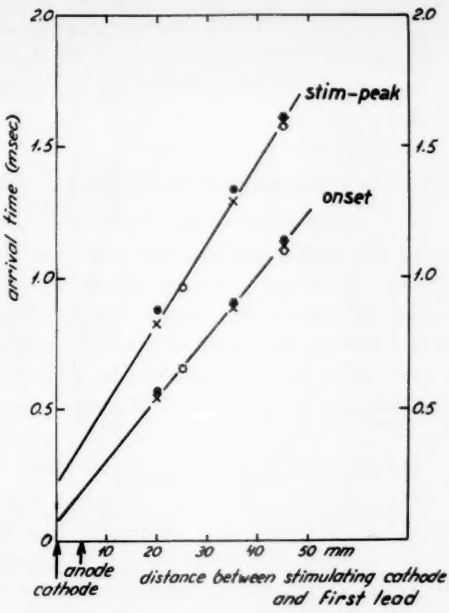


Fig. 7. Conduction time of the action potential over various distances of the nerve (three experiments: ●, ○, ×).

Ordinate: time in msec measured from the stimulation artefact to the onset and to the peak of the potential.

Abscissa: distance on the nerve in mm measured between the stimulating cathode and the first recording electrode.

Conduction velocity of the action potential.

If the onset of the action potential is used for the determination of conduction velocity, temporal dispersion of the action potential does not distort the measurement since the onset always corresponded to the most rapidly conducted impulses (see Fig. 6). Using the peak of the action potential as the point to be measured, the change in the shape of the action potential during propagation caused a shift in the point to be measured which was the greater, the larger the distance between the stimulation and recording points. The peak of the action potential recorded from the postanesthetic section occurred therefore later than in the preanesthetic section so that the conduction time measured was longer (see Fig. 7). The peak was, however, easier to identify than the onset especially when the amplitude was low due to anesthetic effect. Since the object of the anesthetic experiments was to

determine *changes* in conduction velocity the peak of the action potential could be used as long as the distance between stimulating and recording electrodes was the same in the experiments in which conduction velocities were to be compared.

Action potential with various placements of the stimulating cathode and anode.

Action potential amplitude.

When the anode of the stimulating electrode pair was placed closest to the recording electrode, the action potential amplitude was about 20 per cent lower than when the anode was placed distally. This difference in amplitude was due to anode block of some of the fibers between the cathode and the recording electrodes. The proximal electrode of the stimulating pair was therefore the cathode in the anesthetic experiments (see Fig. 2).

Conduction velocity of the action potential.

Stimulating a nerve with the cathode as the distal or as the proximal stimulating electrode, and measuring the conduction time from the stimulus artefact to the action potential, RUSHTON (1949) found that the point of initiation of the nerve impulse did not coincide with the cathode but lay some 3 mm from it extrapolarly. The determination of conduction time from the interval between stimulus artefact and action potential is unsuitable for other reasons:

1) when the stimulus is of high intensity the point of stimulation may lie still further from the cathode.
2) stimulation can only take place at the site of a Ranvier's node (TASAKI 1953). Since the nodes occur at intervals of 0.2–1 mm and are displaced for the single fibers, the site of stimulation extends over a distance of up to 1 mm along the nerve.
3) the latency time, an unknown quantity, occupies some of the time between stimulus and onset of the recorded action potential.

The unsuitability of the stimulus artefact as point of reference is illustrated in Figure 7. Extrapolation to the abscissa of the arrival times as measured to the onsets and to the peaks of the action potentials indicated that stimulation of the nerve occurred extrapolarly and not at the cathode.

Changes in the action potential with various distances between the recording electrodes.

Since the diphasic action potential represents the difference between the two monophasic potentials recorded at the two recording electrodes, the

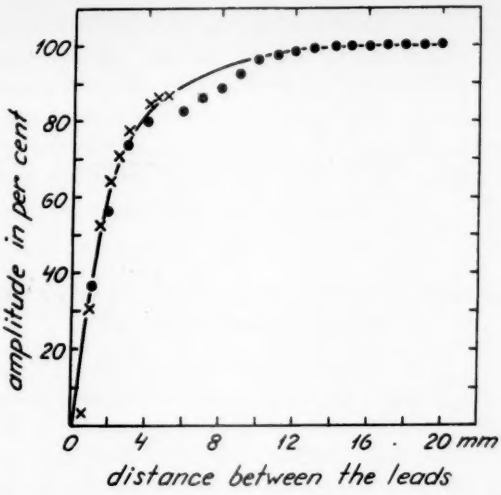


Fig. 8. Amplitude of the nerve action potential as a function of the distance between recording electrodes (two experiments: ×, ●).
Ordinate: amplitude in per cent of the maximum value.
Abscissa: distance between electrodes in mm.

amplitude and shape of the action potential depend on the distance between the recording electrodes. In two experiments this distance was varied between one half and 20 mm and the following changes in amplitude and conduction velocity were found:

Action potential amplitude.

Figure 8 demonstrates that the amplitude increased with increasing distance between the recording electrodes up to a distance of 14 mm. Figure 9 shows action potentials recorded from electrodes at a distance of fourteen and of one mm from each other, as well as the reconstructed monophasic potentials recorded at each of the two recording electrodes (LORENTE DE Nó 1947 and HÅKANSSON 1957).

When the distance between the recording electrodes was 14 mm the monophasic potential recorded at the second recording electrode had just begun when the amplitude of the monophasic potential recorded at the first electrode was maximal, and it did not diminish the front of the diphasic potential. The amplitude of the diphasic potential was equal to that of the monophasic potential.

At electrode distances less than 14 mm a decrease in amplitude occurred

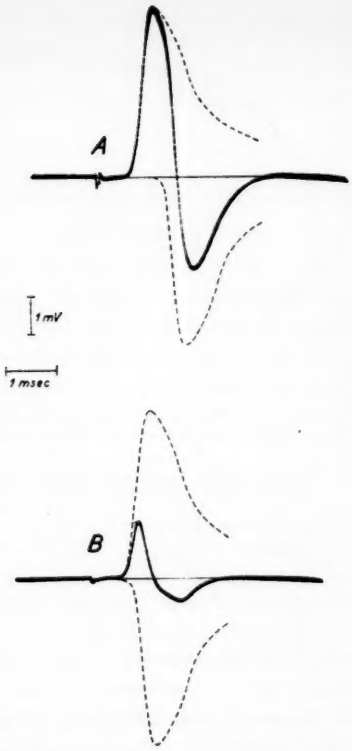


Fig. 9. Diphasic action potentials recorded with (*A*) 14 mm and (*B*) 1 mm between the recording electrodes. The stippled lines are the monophasic potentials recorded at each lead, reconstructed from the diphasic potentials.

since the second monophasic potential had its onset during the front of the potential recorded at the first electrode, as shown for example in Figure 9. The distance of 14 mm corresponding to the front of the monophasic potential is confirmed by measurement of potentials illustrated by LORENTE DE NÓ (1947).

To obtain amplitudes independent of minor variations in distance between the recording electrodes requires that they be separated by more than 14 mm. In view of the limited nerve length available, however, the recording electrodes of a pair were 7 mm apart. According to Figure 8 a variation of 1 mm from this inter-electrode distance would cause a change in amplitude of 2–3 per cent.

Conduction velocity of the action potential.

The conduction velocity was independent of the distance between the recording electrodes when the time interval between the onset of the action potentials was measured. Measuring the time interval between the peaks of the potentials the conduction time was independent of the distance between the electrode pair when this was greater than 6 mm. With smaller inter-electrode distances the conduction time was shorter.

Alterations of the action potential occurring with changes in the resistance between the recording electrodes.

Action potential amplitude.

The resistance between the recording electrodes might alter in the course of an experiment as for example when the layer of fluid surrounding the nerve evaporated. It is known that the amplitude and conduction velocity of the nerve impulse are dependent on the resistance of the medium surrounding the nerve (HODGKIN 1939). It was therefore of interest to ascertain whether a change in resistance between the recording electrodes could influence the amplitude and conduction velocity of the action potential with the set-up used. To this purpose a nerve was laid in the open chamber so that the surrounding fluid layer could evaporate. Figures 10 A, B show examples of the relationship between the resistance between the recording electrodes and the amplitude and conduction velocity of the action potential. The resistance increased rectilinearly in the course of 50 minutes from 9.2×10^3 to 40×10^3 Ohm. The action potential amplitude increased at first proportionally with the resistance and reached a maximum in 30 minutes. Denoting the resistance of the nerve between the recording electrodes R_n, the potential difference over it, V_n, the resistance in the nerve including the fluid shunt R and the potential difference over it V, the rectilinear portion of the curve is given by:

$$\frac{V_n}{V} = \frac{R_n}{R}$$

During the time in which the amplitude increased rectilinearly with resistance, the increase was due solely to a change in the fluid shunt and not in the size of the monophasic potential. In other words the number of active fibers and the amplitude of each single fiber's action potential were unchanged. The nerve was allowed to dry both between stimulating and recording electrodes and between the two recording electrodes. The increase in amplitude could be explained by the change in resistance between the

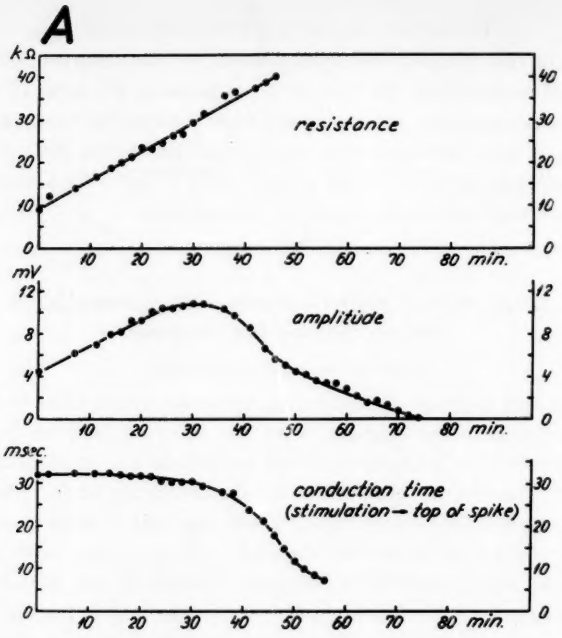


Fig. 10. The influence of the increasing resistance between the recording electrodes.
A. Resistance between recording electrodes, action potential amplitude, and conduction time in the same nerve as a function of time for its exposure to dry air.

Upper ordinate: resistance of the nerve plus the fluid-shunt between recording electrodes (kOhm).

Middle ordinate: action potential amplitude in mV.

Lower ordinate: conduction time measured from the stimulus artefact to the peak of the potential in msec.

Abscissa: time the nerve has been exposed to dry air in minutes.

recording electrodes and was not due to the change in the fluid shunt between stimulating and recording electrodes, as demonstrated by the following experiment:

The resistance between the recording electrodes was kept constant, the external resistance between the stimulating and recording electrodes was diminished from 92.4×10^3 Ohm to 21.1×10^3 Ohm by placing a strip of filter paper moistened with Ringer's solution on the nerve. The amplitude measured without external shunt was 5.1 mV and with the filter paper 5.3 mV, in other words identical within the accuracy of measurement.

It was to be expected that the amplitude be unchanged at the beginning

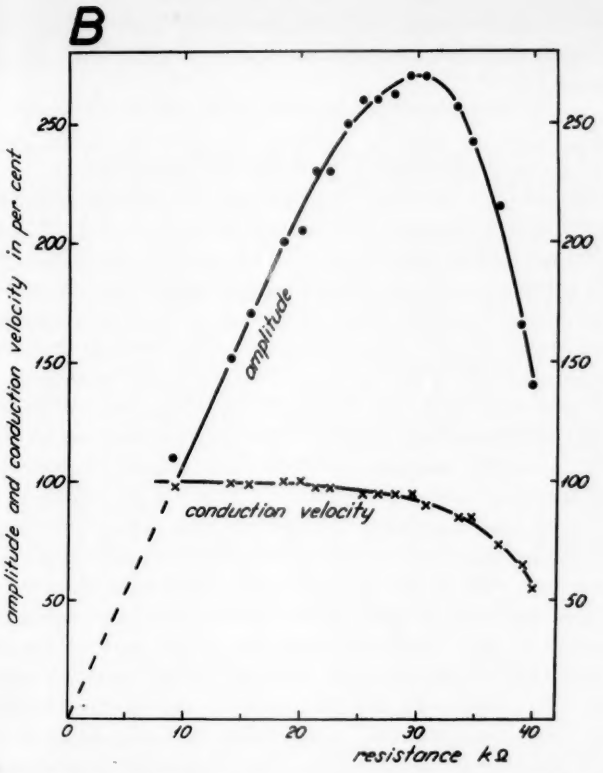


Fig. 10.

B. Action potential amplitude and conduction velocity as a function of the increasing resistance between recording electrodes with the nerve in dry air (cf. A.).

Ordinate: action potential amplitude and conduction velocity in per cent of the values with the nerve in Ringer's.

Abscissa: resistance of the nerve plus fluid shunt in kOhm measured between the two recording electrodes.

of the evaporation experiments and not increase until the fluid layer around the nerve was saturated with ions, i.e. when the specific resistance was constant. However, the amplitude increased from the onset of the evaporation period, probably because the ions diffused into the nerve as their concentration in the fluid increased, so the specific resistance remained constant during the whole period of evaporation and the resistance depended solely on the thickness of the fluid layer. After approximately 25–30 minutes of drying the amplitude fell and after 75 minutes it was close to zero. Since the

condition was not reversible when the nerve was again placed in Ringer's solution, the explanation must be that the nerve fibers were damaged and ceased to conduct.

Conduction velocity of the action potential.

In the period when the amplitude increased rectilinearly as a function of the increase in resistance between the recording electrodes the conduction velocity did not change (Figs. 10 A, B). This was also shown in experiments where the resistance between stimulating and recording electrodes was decreased by means of an extra shunt; the conduction velocity was unchanged when filter paper soaked in Ringer's solution was placed on the nerve.

In the case of single fibers RUSHTON (1937) deduced theoretically, and his deduction was confirmed experimentally by HODGKIN (1939) and KATZ (1947), that the conduction velocity is inversely proportional to the square root of the sum of the inner and outer resistance, so that conduction velocity falls with increased outer resistance. That this did not obtain in the case of whole nerve can be explained by the fact that drying did not alter the resistance in the interfibrillar fluid. When the amplitude began to fall as the evaporation was nearly complete, the conduction velocity also fell towards zero. The fall in conduction velocity might be explained by the disappearance of the interfibrillar fluid, but if this were so the amplitude should increase on account of the increased outer resistance around the single fibers. As mentioned above the amplitude decreased, probably because of damage to the single fibers, causing them to lose potassium and their membrane potential to be diminished. This, too, would involve a reduction in conduction velocity (LORENTE DE NÓ 1947).

Conclusion: Since the fluid shunt surrounding a nerve is dependent on the humidity of the surrounding air, variations in humidity can cause changes in resistance between the recording electrodes and thereby cause changes in the amplitude of the action potential. During long lasting anesthetic experiments it was therefore necessary to measure the resistance at frequent intervals so that the amplitude could be corrected for a change in resistance. The conduction velocity of the action potential did not decrease until the resistance in the fluid shunt increased to more than three times its initial value. A correction of conduction velocity was therefore not requisite in the range of resistance changes which occurred during the experimental conditions.

In addition to direct resistance measurements between the recording elec-

trodes the resistance could be deduced from changes in amplitude at the preanesthetic electrodes. An example of this is described on page 56.

Alterations of the action potential by stretching the nerve.

Since the nerve was slightly stretched between the electrodes in the chamber it was determined whether such stretch could alter the amplitude and conduction velocity of the action potential.

In a series of experiments the nerve was submitted to various degrees of stretch. At each degree of stretch the action potential and the resistance between two recording electrodes were measured. The stretch of the nerve was expressed as per cent of the initial length.

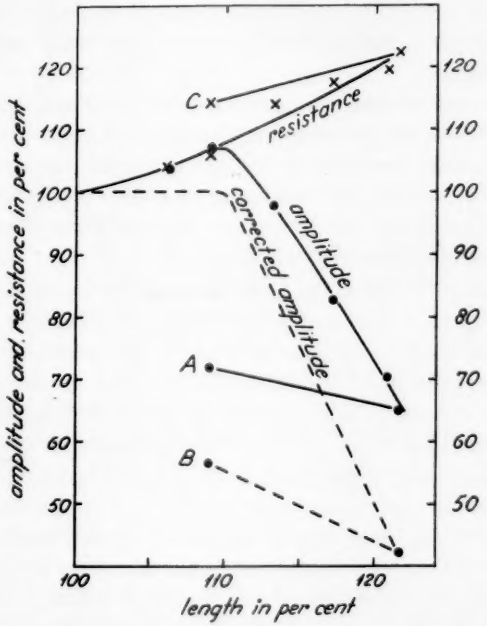


Fig. 11. Action potential amplitude and resistance between two recording electrodes as a function of stretch of the nerve.

Ordinate: amplitude and resistance in per cent of values measured on the unstretched nerve.

Abscissa: length of the nerve in per cent of that of the unstretched nerve.

Points *A* and *C* were obtained when the nerve was allowed to relax after maximum stretch. The broken lines represent the amplitude corrected for the increase in resistance (*B* is the corrected value of *A*).

The action potential amplitude and the resistance between the recording electrodes increased 7–8 per cent with a 10 per cent stretch of the nerve (Fig. 11). Since the amplitude increased proportionately with the resistance between the recording electrodes the increase in amplitude found on stretching the nerve was probably due solely to the increased resistance resulting from the stretch.

When the nerve was stretched more than 10 per cent the amplitude fell while the resistance continued to rise. This is assumed to be due to the fact that some of the nerve fibers were blocked, resulting in a decrease in the amplitude of the whole nerve action potential. On account of the progressive increase in resistance between the recording electrodes, this decrease was still more pronounced than it appeared from the action potential amplitude. Figure 11 shows the reduction in amplitude of the nerve impulse due to blocking of single nerve fibers because of stretch when corrected for the increase in resistance.

If the nerve was allowed to relax after a stretch of about 22 per cent, the length returned to 109 per cent, the resistance to 114.5 per cent, and the corrected amplitude increased to no more than 55 per cent of the initial value (point B, Fig. 11). The decrease in amplitude with stretch is therefore only partially reversible. The irreversible decrease in amplitude may be due to tear of some of the nerve fibers.

The conduction velocity of the action potential was unchanged for stretches of the nerve up to 20 per cent. Thereafter it fell abruptly by about 10 per cent. When the nerve was allowed to relax the conduction velocity increased slightly, but did not return entirely to normal. That the conduction velocity was constant over such large degrees of stretch is in agreement with the findings of BULLOCK et al. (1950) who investigated conduction velocity during stretch of single giant nerve fibers of Lumbricus and Loligo.

Conclusion: The nerve could be stretched by at most 10 per cent without damaging the fibers. With higher degrees of stretch the action potential amplitude fell due to fiber damage. With stretch of less than 10 per cent the amplitude could be corrected for the increased resistance between the recording electrodes, since the amplitude change with stretch of the nerve was proportional to the change in resistance.

The action potential amplitude in experiments on nerves without sheath.

The dissection of the sheath was carried out distal from the union of the 8th, 9th and 10th spinal nerve. To obtain as similar recording conditions as possible in experiments "with and without sheath" the recording electrodes

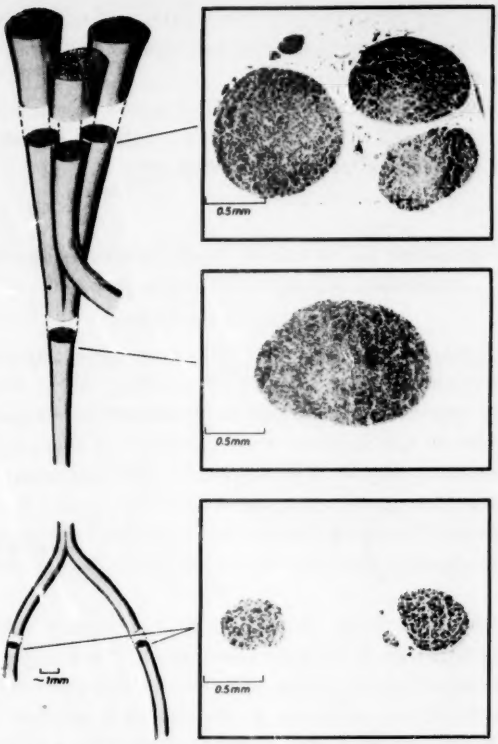


Fig. 12. Histological section through a frog nerve (osmium stain).
Above: at the site of the post-anesthetic recording electrodes in nerves without sheath (8th, 9th and 10th spinal nerves).
Middle: at the site of the post-anesthetic recording electrodes in nerves with sheath (sciatic nerve).
Below: at the point of stimulation (tibial and peroneal nerves).

were placed on a segment of the nerve which had not been stripped of its sheath. The postanesthetic recording electrodes were therefore placed on the three spinal nerves in experiments on nerves without sheath. The resistance between the recording electrodes in these experiments was smaller than in experiments on nerves with intact sheath, where the recording electrodes were placed on the sciatic nerve. This is illustrated on the histological section through the nerve at the two recording sites (Figure 12), where it is seen, that the cross sectional area in experiments "without sheath" was 1.8 times greater than in experiments with intact sheath. The smaller resistance

between the recording electrodes in experiments "without sheath" results in correspondingly lower action potential amplitudes compared with experiments on nerves with sheath.

The mean action potential amplitude in Ringer's solution for 17 nerves "without sheath" was 2.9 $mV \pm 8.6$ per cent (Table 9) in comparison with 5.88 $mV \pm 3.8$ per cent for 63 nerves with sheath.

The effect of temperature and of carbon dioxide tension on the amplitude and conduction velocity of the action potential.

Action potential amplitude.

LORENTE DE NÓ (1947) found that the action potential amplitude was maximal at a temperature of 10–15°C, depending on the temperature to which the frog was accustomed. For temperatures above and below this value the amplitude fell slightly. I have confirmed these findings in the experiment shown in Fig. 13. The amplitude was maximum at 15°C and was about 10 per cent less at 22° and 8°C. Thus, within the temperature range which obtained during an anesthesia experiment the changes in amplitude due to temperature changes were so small that they could be disregarded.

According to LORENTE DE NÓ (1947) it is necessary that the carbon dioxide concentration be 3–5 per cent for normal functioning of the nerve. Carbon dioxide is said to have a specific effect on nerve metabolism. Lack of carbon dioxide results in a decrease in the resting membrane potential and therefore in the amplitude of the action potential. RUDOLPH (1953) found a 20 per cent greater amplitude in the presence of 5 per cent carbon dioxide than in its absence. With the nerve in a humid atmosphere of 97 per cent oxygen and 3 per cent carbon dioxide I found no change in the action potential amplitude over as long a period as 5 hours.

Conduction velocity of the action potential.

The conduction velocity falls with decreasing temperature. It is well known that the conduction velocity changes relatively more with temperature than the amplitude. Values of Q_{10} for the dependence of the conduction velocity on temperature as determined by different investigators are given in Table 1. GASSER's (1931) findings were confirmed in the experiment shown in Figure 14, the Q_{10} being 1.71 at 8° to 18°C and 1.42 at 18–24°C. The velocity fell rectilinearly with decreasing temperature with a slope corresponding to 1 m/sec. per degree centigrade.

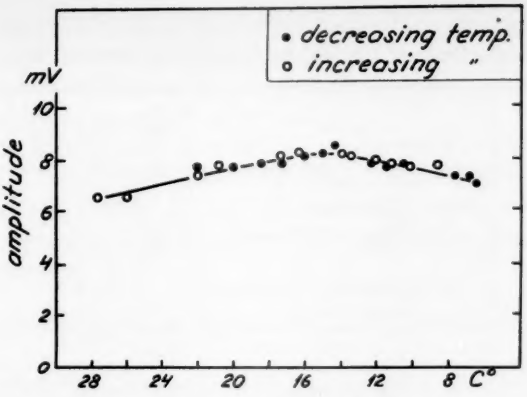


Fig. 13. Action potential amplitude as a function of temperature.

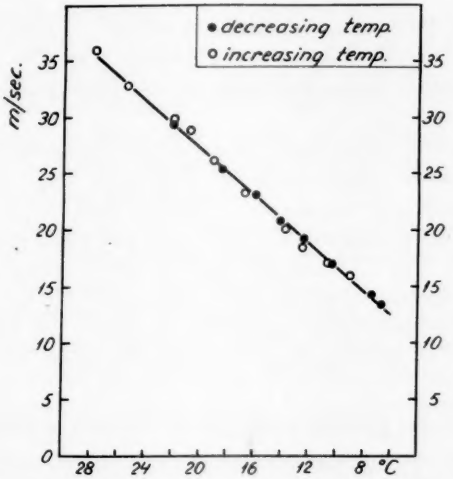


Fig. 14. Conduction velocity of the action potential as a function of temperature (determined for the nerve illustrated in Fig. 13).

Dependence of the nerve action potential on pH.

There was no change in action potential amplitude or conduction velocity when the pH of the Ringer's solution in the test chamber was varied between 5.8 and 7.7. This is in agreement with investigations on single frog nerve fibers (HERTZ 1947). Similarly LORENTE DE NÓ (1947) found for whole frog

Table 1.

Dependence of conduction velocity on temperature.

author	nerve	temperature range °C	Q_{10}
MAXWELL, 1907	pedal nerve (*Ariolimax columbianus*)	10–20	1.55
LUCAS, 1908	sciatic, tibial and sural nerves (*Rana esculenta*)	8–18	1.79
GASSER, 1931	sciatic nerve (green frogs)	8–18 18–24	2.0 1.7

nerve that the activity was not altered by changes in pH between 5.5 and 8. Below pH 3 impulse conduction is blocked. With pH values above 8 spontaneous impulses appear due to the precipitation of calcium (LEHMANN 1937b).

A change in pH is however accompanied by a change in the threshold to stimulation. ADRIAN (1920) found for frog nerve that the threshold to stimulation was 36 per cent higher at pH 5.5 than at pH 9.6. LEHMANN (1937a) found that the threshold in cat nerve rose rectilinearly by a factor of four from pH 8 to 7.2. The fact that the nerve action potential is unchanged with variations in pH may be explained by the fact that the nerve impulse is in any case large enough to depolarize the adjacent not yet activated section of nerve membrane in spite of its elevated threshold.

Dependence of action potential amplitude on the outer sodium concentration.

To obtain isotonic anesthetic solutions the sodium concentration in the Ringer's and anesthetic solutions was decreased from 115 mM (the normal sodium content for frog's plasma) to 75 mM. It was therefore necessary to determine whether the action potential amplitude varied with the outer sodium concentration. Allowing a whole frog nerve to conduct in normal Ringer's and then for $4\frac{1}{2}$ hours in Ringer's solution with 75 mM sodium, no change in amplitude occurred.

The action potential amplitude of the single fiber depends on the outer sodium concentration, the overshoot being proportional with the outer sodium concentration (HUXLEY and STÄMPFLI 1951). With 75 mM sodium

in the bathing fluid, or 64 per cent of the concentration in normal Ringer's, the overshoot is diminished by about 10 mV (HUXLEY and STÄMPFLI 1951). For an action potential amplitude of 116 mV in normal Ringer's this decrease in amplitude amounts to 9 per cent.

The reason that I found no reduction in experiments on whole nerve is probably that the diminished external sodium concentration does not infiltrate the single fibers in the time over which the experiments extend. A whole nerve can conduct for many hours in a sodium-free Ringer's solution without impairment.

FACTORS OF SPECIAL IMPORTANCE DURING LOCAL ANESTHESIA

The influence of the length of the anesthetized portion of nerve.

Experiments with 20 mm of nerve exposed to a high concentration of the local anesthetic showed that the amplitude of the action potential as recorded from the postanesthetic section did not diminish to zero. There persisted a potential of 0.1–0.15 mV, corresponding to 1–2 per cent of the amplitude in Ringer's even after the nerve had been in the anesthetic solution for 50 minutes. The diminution in amplitude to 1–2 per cent occurred in the course of about 6 minutes. To investigate whether the persisting small potential originated from electrotonic conduction over the anesthetized section the following experiment was performed:

The action potential and the electrotonically conducted potential are affected oppositely by changes in the resistance, the action potential amplitude increasing with increased outer resistance.

The outer resistance of the anesthetized section of nerve was changed by placing the nerve first in air and then in Ringer's solution. Since the small persisting potential was about three times greater when the nerve was in Ringer's solution than in air it was an electrotonically conducted potential.

Increasing the length of the anesthetized section of nerve above 20 mm caused a decrease in the amplitude of the electrotonically conducted potential (Fig. 15). At 31 mm it could no longer be discriminated. As a result the experimental chamber was designed to allow 32 mm of nerve to be subjected to the anesthetic and in the subsequent experiments no electrotonic potential was recorded in the postanesthetic section.

The whole section of nerve in the experimental chamber was subjected to the same concentration of the local anesthetic, since the remaining part of the nerve was surrounded by humid air. It is assumed that conduction occurred with constant velocity over the part of the nerve exposed to the local anesthetic.

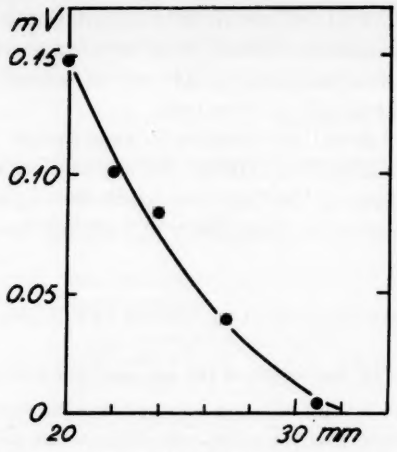


Fig. 15. Size of the electrotonic potential recorded by the post-anesthetic electrodes as a function of the length of the nerve segment blocked by the local anesthetic.
Ordinate: electrotonic potential in mV.
Abscissa: length of the blocked segment of nerve in mm.

LUCAS and coworkers (1917) contrary to this assumption interpreted their anesthetic experiments to indicate that the size and velocity as well as the threshold to stimulation of the nerve impulse diminished or showed decrement along the course of anesthetized nerve. KATO (1924, 1926) claimed on the other hand that in the single fiber either a maximal impulse could be elicited or no impulse at all and that the conduction velocity and threshold were constant along the nerve. Immediately before the nerve was blocked both the size of the impulse and the velocity of its conduction diminished and the threshold to stimulation was elevated.

According to KATO (1924, 1926) LUCAS and coworkers (1917) had neglected to consider the following circumstances:

1) At the boundary between normal and anesthetized nerve a diffusion of the anesthetic occurs into the normal nerve and a diffusion of Ringer's solution into the anesthetized nerve. In this way about 3 mm of nerve on either side of the boundary are subjected to various concentrations of anesthetic. Since LUCAS anesthetized only 4.5 mm of nerve, the conditions along the nerve were not constant. That the conduction velocity, amplitude and threshold varied along the anesthetized section could therefore not be taken as evidence for the existence of decrement in this section.

2) With electrical stimulation the electrotonically conducted potential

influences the threshold. KATO used mechanical stimulation and found that the threshold was constant along the nerve at the same degree of anesthesia.

When only a short section of the nerve is anesthetized, the electrotonic potential can excite the nerve beyond the blocked section. ADRIAN (1912) found for example that the nerve impulse can jump over a section as long as 5 mm. HODGKIN (1937) measured the spread of electrotonic currents over the blocked section. About a tenth of the electrotonic current which spreads from an activated section of nerve is sufficient to activate neighbouring areas. HODGKIN (1937) found a propagated potential when the blocked section of nerve was less than 7–8 mm. An increased outer resistance diminishes the electrotonic spread and therefore also the length of the blocked section over which the action potential can jump. HURYAMA (1941) states that the minimum length of nerve to be blocked is 10 mm to ensure an instantaneous effect of an anesthetic on a single fiber. With smaller distances the blocking time is prolonged, corresponding to the time it takes for the anesthetic to diffuse over the critical section of nerve.

In the investigation reported here a 32 mm section of nerve was exposed to the local anesthetic to 1) prevent activation of the post-anesthetic section of the nerve by electrotonically spread potentials and to 2) prevent distortion of the post-anesthetically recorded action potential by electrotonic spread.

The action potential recorded from the preanesthetic portion of the nerve.

This potential was used as point of reference when determining conduction velocity over the anesthetized section and as an indicator of the general condition of the nerve. To be able to record conduction velocity solely over the section of nerve anesthetized (see however page 36) the proximal recording electrode L_{I_1} (see Fig. 1) was in direct contact with the anesthetic fluid. As a result the shape of the preanesthetic potential changed as anesthesia progressed (see below).

The amplitude of the pre-anesthetic action potential increased with increasing degrees of anesthesia and the action potential became monophasic when block was complete (see Fig. 16). The increase of amplitude may be explained as follows (see also page 39):

Since the second electrode of the preanesthetic pair of recording electrodes was in contact with the anesthetic fluid the potential recorded by it disappeared, resulting in an amplitude recorded solely at the first recording electrode. This amplitude had the same size as the monophasic potential

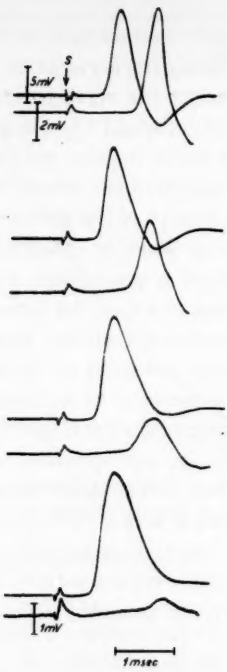


Fig. 16. Pre- and post-anesthetic action potentials with progressing local anesthesia of the nerve.

The pre-anesthetic potential (upper beam) becomes gradually monophasic and its amplitude increases. The post-anesthetic potential (lower beam) diminishes in amplitude with increasing anesthesia. The conduction time from stimulus (*S*) to amplitude peak is gradually prolonged indicating a diminution in conduction velocity in the anesthetized section of the nerve (2 mm between recording electrodes).

(maximal amplitude), even if the distance between the recording electrodes was less than 14 mm. With electrode distances greater than 14 mm no change in the preanesthetic potential amplitude occurred during anesthesia of the nerve. The relationship between the initial and final amplitude of the action potential recorded preanesthetically as a function of electrode distance, is shown in Table 2.

During anesthesia of the nerve the amplitude recorded at the preanesthetic electrode increased rectilinearly as the amplitude at the postanesthetic electrode diminished (Fig. 17). Hence, a measure of the effect of the anesthetic on the nerve could be obtained from the amplitude recorded at the preanesthetic electrodes. Since, however, the amplitude varied by no

Table 2.

Amplitude of the pre-anesthetic diphasic action potential for various distances between the recording electrodes.

electrode distance mm	amplitude in per cent	
	referred to the amplitude of the monophasic pre-anesthetic potential of the blocked nerve*)	referred to the amplitude of the potential measured with an electrode distance exceeding 14 mm**)
1	35	36
2.5	65.5	69
5	81.4	86.5
7	95.1	92.5
14	100	100

*) block at electrode L_{I_2}.
**) values from Fig. 8.

more than about 10 per cent with electrode distances of 7 mm, the uncertainty was large.

By diminishing the distance between the preanesthetic recording electrodes the amplitude recorded from them varied more with anesthesia. With a distance between electrodes of 1 mm the variation was about 65 per cent (Figs. 9, 17). In view of KATO's finding (1924) that the anesthetic diffuses along the nerve, the nerve may with this short interelectrode distance be anesthetized at the first (peripheral) electrode as well causing a reduction in the amplitude of the potential recorded here. With a 7 mm inter-electrode distance diffusion of the anesthetic along the nerve would affect the action potential amplitude only slightly.

In view of the relationship between the pre-and postanesthetic potential amplitudes during anesthesia (Fig. 17), the amplitude of the preanesthetic potential allows the results from the postanesthetic recording to be checked. At the time when the latter had fallen to zero the amplitude at the preanesthetic recording must have increased to its maximum. If the preanesthetic potential amplitude was less than maximum, the nerve had suffered damage. If the amplitude of the preanesthetic potential was greater than its maximum, the fluid layer around the nerve must have evaporated, resulting in

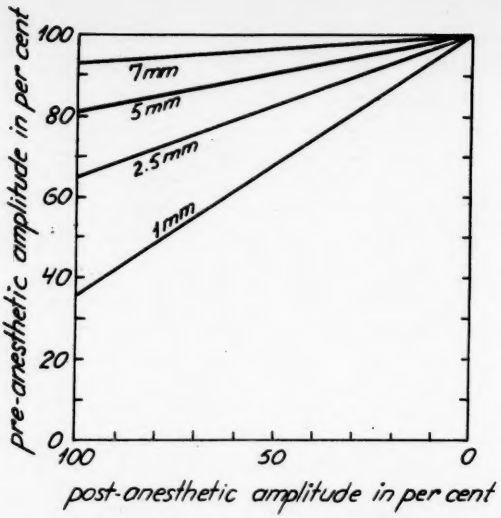


Fig. 17. Increase in action potential amplitude at the pre-anesthetic recording as anesthesia progresses for various distances between recording electrodes as indicated on the curves. The amplitude of the post-anesthetic potential indicates the degree of anesthesia. Ordinate: amplitude of the pre-anesthetic potential in per cent of the final value. Abscissa: amplitude of the post-anesthetic potential in per cent of the value in Ringer's.

an increased resistance between the recording electrodes and an increased amplitude.

From the amplitude of the preanesthetic potential it was possible to calculate changes in the resistance between the recording electrodes. In experiment 162 for example the amplitude at the preanesthetic electrode was 14.7 mV at the beginning of the experiment with the nerve in Ringer's and with a 7 mm inter-electrode distance. At the stage of anesthesia when the amplitude of the postanesthetic potential was 50 per cent the preanesthetic potential amplitude was 18.25 mV. This increase in amplitude could arise from partial blocking of the nerve at the proximal electrode of the preanesthetic electrode pair plus the effect of increase in resistance between the recording electrodes. With the nerve in Ringer's the amplitude should be 93 per cent of its maximum value (Fig. 17) when the electrodes were separated by 7 mm. The maximum amplitude must therefore have been 15.8 mV. When the postanesthetic amplitude was 50 per cent, the preanesthetic amplitude according to Figure 17 should be 96.5 per cent of its maximum, i.e. 15.3 mV. The actually found value of 18.25 mV was therefore 19 per cent greater than to be expected. The resistance between the preanesthetic recording electrodes was measured with the nerve in Ringer's to be 26.6×10^3 and at 50 per cent amplitude to be 31.4×10^3 Ohm, i.e. the resistance increased 18 per cent, and could account for the increase in amplitude of the preanesthetic potential (cf. p. 41).

Conduction velocity of the action potential.

The change in action potential shape at the preanesthetic recording electrodes during anesthesia of the nerve did not influence the onset of the potential. The time from the point of stimulation to the peak of the potential was increased on account of the disappearance of the positive portion of the action potential recorded at the proximal recording electrode. In experiment no. 79 for example with recording electrodes 7 mm apart this time increased by 0.015 msec from the beginning of the experiment to full blockage of the nerve. With a conduction velocity of 30 m/sec this corresponded to 0.45 mm of nerve. When the conduction velocity was measured over 39 mm of nerve the error was 1.2 per cent.

The action potential recorded from the postanesthetic portion of the nerve.

The interelectrode distance was of no importance for the *amplitude* of the postanesthetic action potential during anesthesia of the nerve, since the nerve was blocked at the first recording electrode.

With respect to *conduction velocity* during anesthesia it is shown on page 119 that the nerve conducted at normal velocity when the impulse had passed the anesthetized section of nerve.

EXPERIMENTAL METHOD ADOPTED FOR MEASUREMENT OF ACTION POTENTIAL AMPLITUDE AND CONDUCTION VELOCITY

On the basis of the considerations and experiments performed with respect to the influence of various factors on action potential amplitude and conduction velocity the following experimental method was adopted:

1. The stimulus had a duration of 0.15 msec and an intensity 2–3 times threshold of maximum action potential amplitude in order to allow recording of action potentials from α, β and γ fibers of the A group (see p. 34).

2. The nerve was stimulated at its distal end so that all activated fibers passed uninterruptedly throughout the anesthetized section of nerve (see p. 36).

3. The proximal stimulating electrode was used as cathode to avoid a reduction in amplitude on account of anodal block (see p. 38).

4. The distance between the ground electrode and the closest recording electrode was more than 6 mm to avoid stimulus artefact (see p. 20).

5. To ensure independence of amplitude from minor variations in distance between the recording electrodes an inter-electrode distance of 7 mm was used. A variation of 1 mm from this inter-electrode distance resulted in a variation in amplitude of 2–3 per cent (see p. 40).

6. The length of the anesthetized section of nerve should be greater than 10 mm to prevent the nerve impulse from "jumping over" the anesthetized section. To avoid recording an electrotonic potential at the postanesthetic recording site the anesthetized section of nerve was 32 mm (see p. 53).

7. Since the amplitude of the action potential increased with increased resistance between the recording electrodes (see p. 44) resistance was measured frequently. If the resistance between the recording electrodes changed during the experiment, the amplitude was corrected accordingly since it was directly proportional with resistance.

The variation in resistance between the recording electrodes, which occurred in these experiments, did not affect the conduction velocity.

8. Since the amplitude of the action potential was only slightly dependent on temperature (see p. 48) a correction for the small variations in temperature which could occur in these experiments was not considered necessary. The conduction velocity was corrected for variations in temperature since a temperature change of 1°C corresponded to a conduction velocity change of 1 m/sec (see p. 48). All conduction velocities were corrected to 22°C.

9. With moderate stretch of the nerve a change in action potential amplitude occurred on account of the increased resistance between the recording electrodes (see p. 46). The degree of stretch was not allowed to exceed 10 per cent and no alterations during the experiment were allowed, unless the resistance was recorded and corrected for. The conduction velocity was not dependent on the degree of stretch as long as stretch did not exceed 20 per cent.

10. The chamber was bubbled with 97 per cent oxygen and 3 per cent carbon dioxide (see p. 31).

11. The chamber was maintained moist to prevent the nerve from drying (see p. 23).

Chapter 4

THE ANESTHETIC EFFECT OF XYLOCAINE AND PROCAINE ON PERIPHERAL NERVE

The purpose of the investigations described in this chapter was to investigate the time course of xylocaine and procaine anesthesia in a peripheral nerve. Considering that a local anesthetic must diffuse from the outer fluid into the nerve, the time course of anesthesia must be assumed to depend on the ratio between the minimum concentration and the outer concentration of the active component of the anesthetic. It is assumed that the active component is the uncharged base portion of the anesthetic (Gros 1910, Trevan and Boock 1927, Gardner, Semb and Graham 1934 and Ehrenberg 1948). However Skou (1954), using muscle contraction as indicator, found that the minimum concentration of procaine expressed as uncharged base increased with increasing pH, and for this reason he doubts that the base alone is the anesthetically active portion.

In a series of experiments the minimum concentration of xylocaine and procaine at various pH levels was determined with the action potential as indicator. In another series of experiments the time course of anesthesia with procaine and xylocaine was determined with various concentrations of base. To contribute to the question as to whether it is the uncharged base which is the active component, experiments have been compared in which the concentration of base was constant while pH differed, and when the nerve was subjected to the same hydrochloride concentration at increasing pH levels with corresponding increase in concentration of base and decrease in concentration of acid.

Finally, to investigate the importance of the nerve sheath for the time course of anesthesia, experiments were carried out on nerves without sheath.

RESULTS

Minimum concentration of xylocaine and procaine.

The minimum concentration was determined in the studies presented here as the smallest concentration of base which blocked a nerve exposed to it

Table 3.

Minimum concentration of xylocaine base at different pH.

xylocaine base concentration (C_B) mM	pH	number of experiments	duration of experiments hours	final action potential in per cent of the amplitude in Ringer's
0.23	7.10	12	2	0
0.15	7.20	10	8	0
0.13	7.17	10	6	0
0.10	7.10	10	7	0.3–8
0.10	6.0	10	6	0
0.06	6.0	10	6	0
0.04	6.0	10	8	4 nerves: 0 5 nerves: 0.2–2 1 nerve: 10

From these experiments the minimum concentration for xylocaine base was estimated to be: 0.11 mM at pH 7.10 and 0.04 mM at pH 6.0.

Table 4.

Minimum concentration of procaine base at different pH.

procaine base concentration (C_B) mM	pH	number of experiments	duration of experiments hours	final action potential in per cent of the amplitude in Ringer's
0.026	7.65	10	7.5	4 nerves: 0 4 nerves: 3, 4, 6, 9 2 nerves: 20–35
0.030	7.18	10	6	0
0.021	7.08	9	7	4 nerves: 0 5 nerves: 0.3–6
0.014	7.15	10	6	24–71 %
			19	3 nerves: 13, 34, 40 7 nerves: 0.9–5
0.013	6.12	10	6	6 nerves: 0 4 nerves: 13, 19, 84, 96

From these experiments the minimum concentration for procaine base was estimated to be:

0.026 mM at pH 7.65
0.021 mM at pH 7.08
0.013 mM at pH 6.12

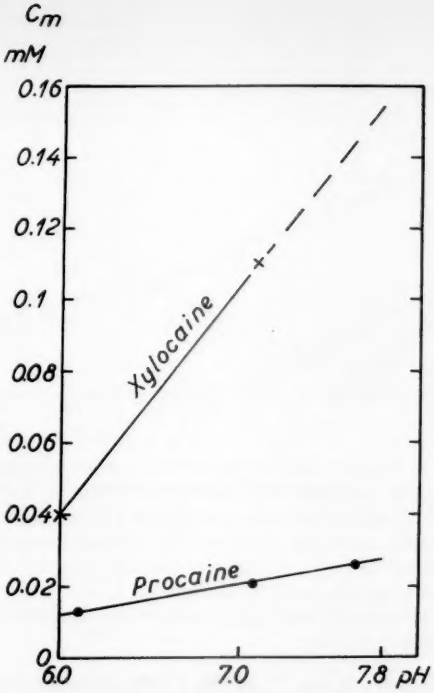


Fig. 18. Minimum concentration of procaine and xylocaine base as a function of pH. Ordinate: minimum base concentration of procaine and xylocaine in mM. Abscissa: pH.

for 6–8 hours (see page 24 and 76). After this time it must be assumed that the outer concentration of anesthetic was equilibrated with the interfibrillar fluid. The minimum concentration was determined at various pH values. The results are presented in Table 3 and 4 and Figure 18. The minimum concentration of procaine was 4–5 times lower than of xylocaine and the minimum concentration for both local anesthetics diminished with diminishing pH of the solution.

The time course of xylocaine and procaine anesthesia.

The effect of xylocaine and procaine on isolated frog nerve was followed using the gradual diminution in amplitude of the nerve action potential as measure of the development of anesthesia.

The concentration of xylocaine and procaine hydrochloride varied between 1 and 40 mM. The pH values in the experiments varied between 5.67 and 7.8. For the 47 experiments with xylocaine 87 per cent of the pH values lay between 6.75 and 7.55 with a mean value of 7.08. In the case of procaine

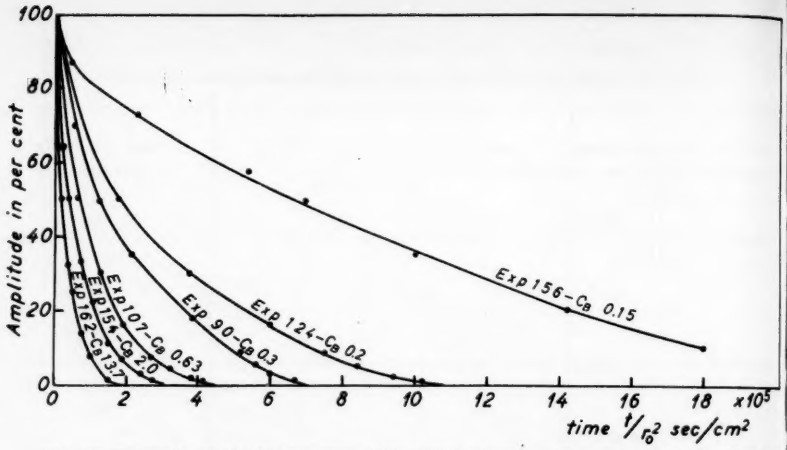


Fig. 19. Examples of the time course of the effect of various concentrations of xylocaine base on action potential amplitude. The experiments illustrate that anesthesia occurs more rapidly with increasing concentration (temperature range: 21–23.9°C*)).
Ordinate: action potential amplitude as per cent of the amplitude in Ringer's.
Abscissa: time expressed as t/r_0^2 to compensate for differences in nerve radius (r_0); t is the time after application of the anesthetic.
The numbers on the curves indicate the number of the experiment and the concentration of xylocaine base (C_B) in mM.

the pH range was 6.8–7.8, but 11 of the 17 experiments were carried out at pH 7.5 and 7.8. To ensure that the pH of the nerve did not alter in the course of the experiment, all nerves were placed for one hour before the experiment in Ringer's solution with the same pH as that of the anesthetic solution to be used. The experiments were carried out at 18-26°C.

Examples of the effect of various concentrations of xylocaine base are illustrated in Figure 19. The amplitude began to fall immediately when the nerve was placed in the anesthetic solution. The amplitude diminished most per unit time just after the application of xylocaine and the diminution then progressed more slowly to full block. The course of anesthesia was accelerated when the base concentration was increased.

The time course of diminution of the action potential amplitude during anesthesia was described by the time at which the amplitude reached half its original value ($t_{.05}$) and "blocking time" ($t_{0.01}$) which is the time required for the amplitude to fall to 1 per cent of its original value. The time to half amplitude of the action potential and to blocking became shorter with increasing concentration of base (Figures 20, 21).

*) As to the influence of difference in temperature cf. p. 74.

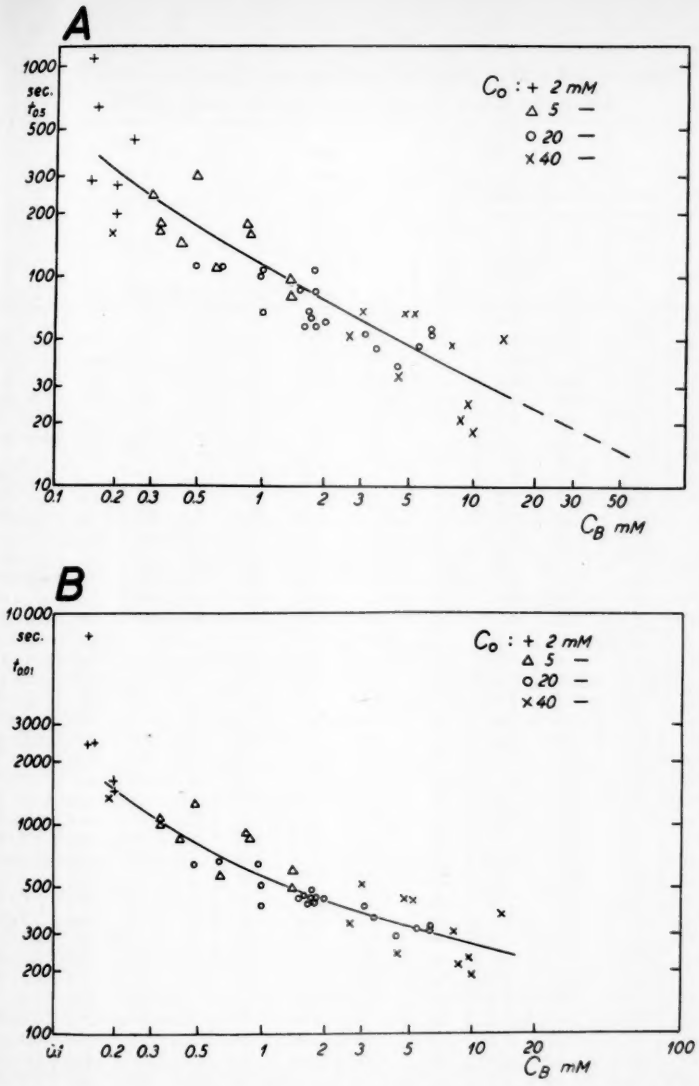


Fig. 20. Time to (*A*) half action potential amplitude ($t_{0.5}$) and (*B*) to block ($t_{0.01}$) as a function of xylocaine base concentration in experiments with different hydrochloride concentrations (C_o) (temperature range: 20–25.5°C, see footnote p. 000).

Ordinate: seconds after application of the anesthetic (logarithmic scale).

Abscissa: xylocaine base concentration (C_B) in mM (logarithmic scale).

Table 5.

Time to half action potential amplitude ($t_{0.5}$) and to block ($t_{0.01}$) in experiments with xylocaine (nerves with sheath).

experiment no.	temperature °C	nerve radius (r_0) mm	action potential amplitude in Ringer's mV	hydrochloride concentration (C_0) mM	pH	base concentration (C_B) mM	$t_{0.5}$ sec	$\frac{t_{0.5}}{r_0^2}$ sec/cm²	$t_{0.01}$ sec	$\frac{t_{0.01}}{r_0^2}$ sec/cm²
132	23.1	0.39	7.3	1	6.70	0.05	720	46.1×10^4	—	—
156	22.5	0.39	11.0	2	6.88	0.15	108	69.7×10^4	7800	50.3×10^5
115	23.6	0.41	3.6	2	6.88	0.15	285	16.9×10^4	2440	14.5×10^5
123	21.5	0.40	9.2	2	6.90	0.16	630	39.4×10^4	2490	15.6×10^5
102	22.4	0.41	5.0	40	5.67	0.19	160	9.5×10^4	1340	7.9×10^5
124	23.9	0.40	4.8	2	7.00	0.2	270	16.9×10^4	1633	10.2×10^5
126	28.5	0.38	5.6	2	7.02	0.2	198	13.6×10^4	1452	10.0×10^5
116	21.8	0.38	4.7	2	7.09	0.24	441	30.4×10^4	—	—
90	23.1	0.43	7.4	5	6.77	0.30	240	12.9×10^4	1250	6.7×10^5
92	23.4	0.42	3.6	5	6.84	0.33	165	9.3×10^4	1008	5.7×10^5
136	24.2	0.45	7.2	5	6.84	0.33	178	8.6×10^4	1097	5.3×10^5
91	23.1	0.45	5.7	5	6.92	0.41	148	7.3×10^4	840	4.1×10^5
93	23.4	0.57	5.8	5	6.98	0.48	300	9.4×10^4	1250	3.9×10^5
106	23.4	0.39	4.5	20	6.38	0.48	105	6.9×10^4	635	4.1×10^5
107	22.3	0.40	4.9	20	6.50	0.63	105	6.6×10^4	652	4.1×10^5
86	25.1	0.38	3.9	5	7.11	0.63	110	7.6×10^4	560	3.9×10^5
94	23.3	0.43	6.1	5	7.28	0.85	178	9.5×10^4	807	4.3×10^5
84	23.1	0.43	4.5	5	7.29	0.87	150	8.1×10^4	860	4.6×10^5
99	23.6	0.35	6.4	20	6.70	0.96	90	7.5×10^4	635	5.3×10^5
157	22.3	0.40	5.1	20	6.70	1.0	85	5.3×10^4	507	3.1×10^5
158	24.0	0.41	6.4	20	6.70	1.0	60	3.6×10^4	402	2.4×10^5
87	25.2	0.38	2.5	5	7.54	1.4	80	5.5×10^4	495	3.4×10^5
88	25.2	0.43	4.5	5	7.55	1.4	96	5.2×10^4	590	3.2×10^5
100	24.2	0.37	6.1	20	6.89	1.5	76	5.5×10^4	440	3.2×10^5
101	23.6	0.38	4.8	20	6.92	1.59	57	4.0×10^4	458	3.3×10^5
76	24.0	0.44	6.6	20	6.93	1.62	67	3.4×10^4	414	2.1×10^5
95	22.6	0.43	1.8	20	6.95	1.74	105	5.8×10^4	480	2.7×10^5
96	21.9	0.38	4.4	20	6.97	1.78	82	5.8×10^4	450	3.1×10^5
103	21.5	0.36	4.6	20	7.01	1.8	57	4.4×10^4	442	3.4×10^5
128	22.3	0.42	5.5	20	6.99	1.8	46	2.6×10^4	444	2.5×10^5
154	22.7	0.40	9.5	20	7.02	2.0	60	3.7×10^4	440	2.7×10^5
109	20.0	0.39	5.4	40	6.83	2.63	51	3.3×10^4	337	2.2×10^5
159	21.3	0.44	9.6	40	6.90	3.0	67	3.5×10^4	510	2.6×10^5
104	21.6	0.38	7.7	20	7.23	3.1	53	3.7×10^4	405	2.8×10^5
79	22.8	0.39	7.8	20	7.30	3.47	45	2.9×10^4	355	2.3×10^5
108	22.8	0.40	4.2	20	7.44	4.36	37	2.3×10^4	292	1.8×10^5
130	24.1	0.37	5.3	40	7.09	4.47	31	2.3×10^4	241	1.8×10^5
110	21.8	0.49	4.5	40	7.12	4.78	65	2.7×10^4	442	1.8×10^5
111	21.3	0.44	4.7	40	7.16	5.13	66	3.4×10^4	429	2.2×10^5
161	22.2	0.46	6.3	20	7.57	5.5	46	2.2×10^4	330	1.6×10^5
98	23.7	0.42	6.9	20	7.66	6.3	52	2.9×10^4	310	1.7×10^5
97	22.7	0.42	4.8	20	7.64	6.31	56	3.2×10^4	312	1.8×10^5
160	21.2	0.44	7.5	40	7.40	8.1	47	2.4×10^4	306	1.6×10^5
112	22.0	0.38	3.1	40	7.44	8.71	21	1.5×10^4	216	1.5×10^5
114	23.1	0.42	4.3	40	7.49	9.55	24	1.4×10^4	235	1.3×10^5
113	22.3	0.38	3.7	40	7.52	10.0	18	1.2×10^4	192	1.3×10^5
162	21.0	0.43	8.2	40	7.72	13.7	50	2.7×10^4	369	2.0×10^5

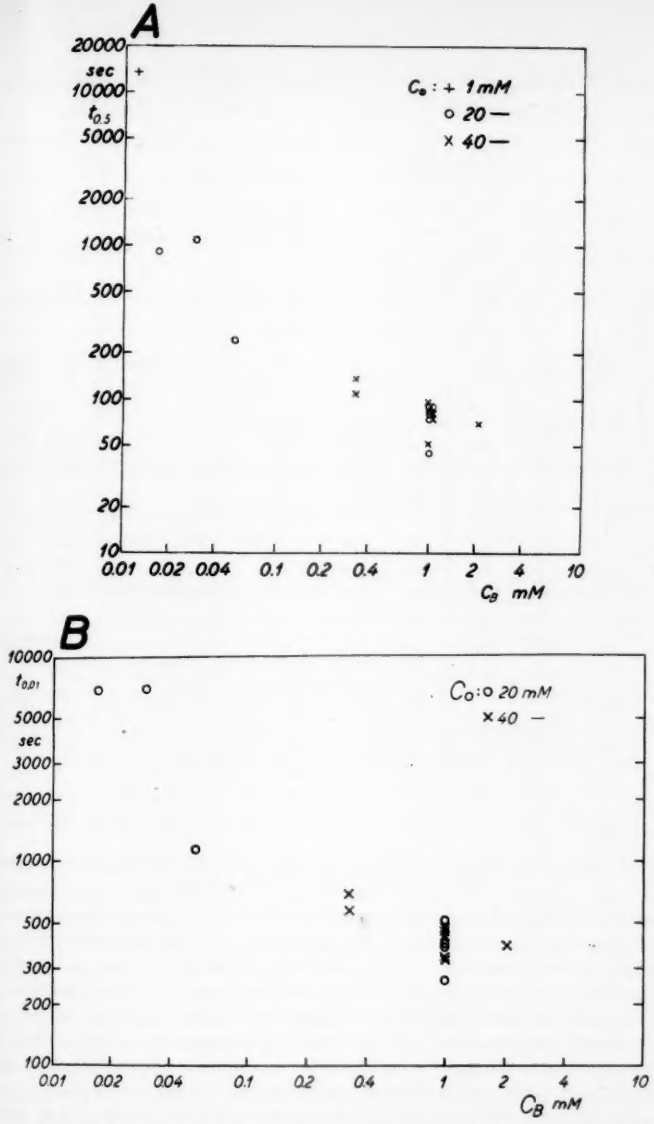


Fig. 21. Time to (*A*) half action potential amplitude ($t_{0.5}$) and (*B*) to block ($t_{0.01}$) as a function of procaine base concentration in experiments with different hydrochloride concentrations (C_0) (temperature range: 18.6–25.2° C, see footnote p. 62).
Ordinate: seconds after application of the anesthetic (logarithmic scale).
Abscissa: procaine base concentration (C_B) in mM (logarithmic scale).

Table 6.

Time to half action potential amplitude ($t_{0.5}$) and to block ($t_{0.01}$) in experiments with procaine (nerves with sheath).

experiment no.	temperature °C	nerve radius (r_0) mm	action potential amplitude in Ringer's mV	hydrochloride concentration (C_0) mM	pH	base concentration (C_B) mM	$t_{0.5}$ sec	$\frac{t_{0.5}}{r_0^2}$ sec/cm²	$t_{0.01}$ sec	$\frac{t_{0.01}}{r_0^2}$ sec/cm²
170	24.0	0.41	4.2	1	7.18	0.012	13800	8.2×10^6	—	—
139	22.2	0.38	6.4	20	6.00	0.017	900	6.4×10^5	8500	5.9×10^6
151	22.3	0.45	5.6	20	6.20	0.030	1000	4.9×10^5	7000	3.5×10^6
138	22.8	0.47	6.1	20	6.50	0.054	240	1.1×10^5	1127	5.2×10^5
153	22.3	0.43	7.2	40	7.00	0.32	135	7.5×10^4	666	3.7×10^5
135	24.5	0.45	7.4	40	7.00	0.33	97	4.8×10^4	568	2.9×10^5
140	25.0	0.35	4.8	20	7.80	1.0	45	3.7×10^4	261	2.2×10^5
141	21.5	0.36	7.9	20	7.80	1.0	75	5.8×10^4	450	3.5×10^5
145	18.6	0.38	8.0	20	7.80	1.0	83	5.9×10^4	385	2.7×10^5
149	23.7	0.41	8.8	20	7.80	1.0	82	4.9×10^4	462	2.8×10^5
150	23.9	0.45	7.1	20	7.80	1.0	81	3.9×10^4	393	1.9×10^5
142	22.0	0.36	6.9	40	7.50	1.0	55	4.2×10^4	340	2.6×10^5
143	21.9	0.38	5.8	40	7.50	1.0	81	5.7×10^4	475	3.3×10^5
147	25.7	0.40	5.3	40	7.55	1.1	85	5.3×10^4	438	2.7×10^5
148	26.0	0.38	—	40	7.50	1.0	68	4.7×10^4	321	2.3×10^5
146	25.3	0.43	6.2	20	7.80	1.0	90	5.0×10^4	510	2.8×10^5
137	23.2	0.39	6.5	40	7.80	2.0	70	4.5×10^4	390	2.6×10^5

Variability of the experimental results.

Xylocaine. In Figure 20 the curves are drawn around which the experimental values for the time to half and to 1 per cent of the initial action potential amplitude are grouped. In 3 ranges of base concentration the spread of the individual values around the mean was calculated. The standard deviation and the mean error are listed in Table 7. The variability was least when the base concentration was in the range 1.0–4.0 mM and the blocking time showed less spread than the time to half amplitude of the action potential. In Figure 20 no correction has been made for the variations in pH and the resultant variations in minimum concentration. Furthermore, no correction has been made for differences in the nerve diameter which can give rise to variations, in that BENNETT et al. (1942) and EHRENBERG (1948) found that the blocking time was proportional to the square of the nerve radius. To eliminate the effect of these factors on the variability Figures 22 A, B show the corrected time (t/r_0^2, when r_0 is the radius of the nerve) as a function of the ratio between base and minimum concentration, making use of the minimum concentrations

Table 7.

Standard deviation (S.D.) and mean error (ε) of the time to half amplitude and to block in per cent of the mean value in different base concentration ranges (for xylocaine cf. Figs. 20 and 22, for procaine cf. Table 6).

	base concentration range mM	number of experiments	$t_{0.5}$		$t_{0.01}$	
			S.D.	$\pm\varepsilon$	S.D.	$\pm\varepsilon$
xylocaine	0.2–0.96	14–13	37	10	17	5
	1.0–4.0	16	24	6	13	3
	4.0–13.7	12	40	10	20	6
procaine	1.0	10	21	7	19	6
	C_B/C_m range	number of experiments	$t_{0.5}/r_0^2$		$t_{0.01}/r_0^2$	
			S.D.	$\pm\varepsilon$	S.D.	$\pm\varepsilon$
xylocaine	3.4–12*)	15	20	5	19	5
	15–90	24	26	5	17	3.5
procaine	37–40	10	14	4	17	6

*) Experiment no. 102 (with extremely low pH) at $C_B/C_m = 10.5$ is omitted.

at different pH shown in Figure 18. The spread of the corrected values (Table 7) did not differ significantly from that of the uncorrected values. The relatively large spread is probably due to biological variations from frog to frog. It may be mentioned that small variations in base concentration in the range near the minimum concentration result in large changes in the time course of anesthesia. Such variations in base concentration can occur even with slight alterations in the pH of the solution. For example on increase in pH of 0.1 unit can increase the base concentration from 0.15 to 0.19 mM and thereby increase the time to half amplitude ($t_{0.5}$) by a factor of 2 (Figure 20 A). With higher base concentrations some of the variability can perhaps be ascribed to uncertainties in the determination of the moment of application of the local anesthetic in that the action potential amplitude diminishes rapidly after its application.

In the case of **procaine** the number of experiments allowed evaluation of the spread solely at the concentration 1.0 mM procaine base. The standard deviation of the individual values and the mean error of $t_{0.5}$, $t_{0.01}$ and $t_{0.5}/r_0^2$, $t_{0.01}/r_0^2$ are shown in Table 7. It is seen that the spread was less when differences in the radius of the nerve were corrected for.

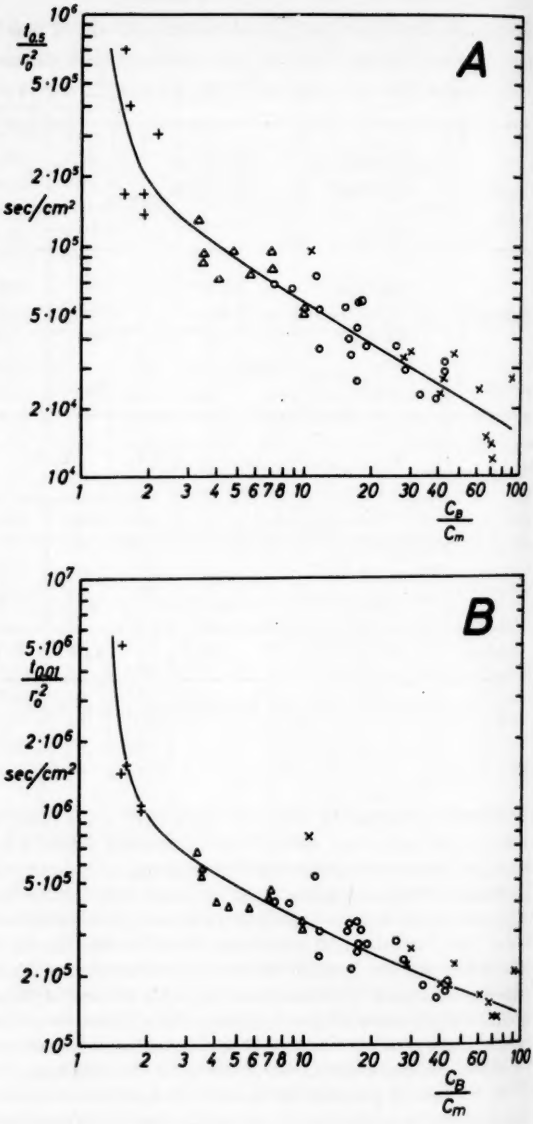


Fig. 22. The time to (*A*) half action potential amplitude and (*B*) to block for different concentrations of xylocaine base, corrected for differences in nerve radius and pH (temperature range: 20–25,5°C, see footnote p. 62).

Ordinate: t/r_0^2 where t is the time in seconds after application of the anesthetic and r_0 the nerve radius in centimeters (logarithmic scale).

Abscissa: xylocaine base concentration (C_B) in units of the minimum concentration (C_m) (logarithmic scale).

The minimum concentration used is that found at the pH of the individual experiments. The symbols indicate hydrochloride concentrations: + = 2 mM, △ = 5 mM, ○ = 20 mM, × = 40 mM.

Table 8.

Time to half action potential amplitude ($t_{0.5}$) and to block ($t_{0.01}$) at different xylocaine base concentrations (C_B/C_m) as a function of the xylocaine acid concentration.

experiment no.	hydrochloride concentration (C_0) mM	pH	acid concentration (C_{BH^+}) mM	base concentration (C_B) mM	minimum concentration *) (C_m) mM	C_B/C_m	$\frac{t_{0.5}}{r_0^2}$ sec/cm²	$\frac{t_{0.01}}{r_0^2}$ sec/cm²
84	5	7.29	4.13	0.87	0.12	7.2	8.1×10^4	4.6×10^5
94	5	7.28	4.15	0.85	0.12	7.0	9.5×10^4	4.3×10^5
106	20	6.38	19.52	0.48	0.07	7.4	6.9×10^4	4.1×10^5
104	20	7.23	16.9	3.1	0.12	25.8	3.7×10^4	2.8×10^5
79	20	7.30	16.53	3.47	0.12	28.2	2.9×10^4	2.3×10^5
109	40	6.83	37.37	2.63	0.09	28.2	3.3×10^4	2.2×10^5
159	40	6.90	37.0	3.0	0.10	30.0	3.5×10^4	2.6×10^5
161	20	7.57	14.5	5.5	0.14	39.3	2.2×10^4	1.6×10^5
98	20	7.66	13.7	6.3	0.15	42.8	2.9×10^4	1.7×10^5
97	20	7.64	13.69	6.31	0.15	43.2	3.2×10^4	1.8×10^5
130	40	7.09	35.53	4.47	0.11	40.6	2.3×10^4	1.8×10^5
110	40	7.12	35.22	4.78	0.11	42.7	2.7×10^4	1.8×10^5
111	40	7.16	34.87	5.13	0.11	46.7	3.4×10^4	2.2×10^5

*) Values from Fig. 18.

Base concentration of the anesthetic versus the acid concentration as anesthetic agent.

Assuming that it is solely the base which is the active anesthetic factor, the time course of anesthesia would be determined by the ratio C_B/C_m. To test this assumption the time course of anesthesia can be compared in experiments where C_B/C_m was constant but where the concentration of xylocaine acid differed. The result of such a comparison is given in Table 8. In the case of xylocaine the base concentration was 7, 28 and 42 times the minimum concentration and at each of these levels the acid concentration was varied 2–5 times. An increase in acid concentration by up to 4.7 times did not affect

the time course of anesthesia as long as C_B/C_m was constant. On the other hand an increase of C_B/C_m by 4 times with unaltered acid concentration reduced the half and blocking time by more than 40 per cent. Hence, in the experiments reported here the course of anesthesia was determined primarily by the concentration of base.

In the case of procaine, too, the course of anesthesia was a function of the base concentration (Fig. 21) though the findings were less clear cut than with xylocaine, since in the main the procaine experiments were carried out at base concentrations such that slight changes could influence the time course only slightly.

The time course of xylocaine anesthesia in nerves without sheath.

The nerve sheath was removed over that portion of the nerve to be exposed to the anesthetic (p. 46) and the reduction in action potential amplitude after exposure to various concentrations of xylocaine base was followed.

The time course of anesthesia in nerves without sheath was qualitatively the same as in nerves with sheath. Immediately after application of the anesthetic the amplitude fell rapidly, and this reduction in amplitude continued at a slower rate to block. The results of 17 experiments on nerves without sheath are listed in Table 9.

With increasing base concentration the amplitude of the action potential diminished more rapidly for concentrations between 0.1 and 1.6 mM xylocaine base. In the concentration range 1.6–15.5 mM the time to half amplitude and the blocking time were largely unaltered.

The spread of the experimental results was calculated from 8 experiments in which the xylocaine base concentration was 1.6–2.3 mM. The standard deviation for half-time and blocking time was 26 per cent and 29 per cent and the mean error was $\pm$ 9 per cent and $\pm$ 10 per cent. The standard deviations for $t_{0.5}/r_0^2$ and $t_{0.01}/r_0^2$ were 40 and 35 per cent respectively, i.e. greater than the values uncorrected for the nerve thickness. The explanation may be that the diameter of sheathless nerves increased when they were placed in Ringer's solution (TRUANT and LANZONI 1952, FRANKENHAEUSER and NYSTRÖM 1954).

Comparison of the time course of anesthesia in nerves with and without sheath.

No systematic experiments were carried out on the same nerve with and without sheath since preliminary experiments had demonstrated that it was impossible to wash the nerve completely free of anesthetic substance after the first anesthesia.

The importance of the nerve sheath for the time course of anesthesia is

Table 9.

Time to half action potential amplitude ($t_{0.5}$) and to block ($t_{0.01}$) in experiments with xylocaine (nerves without sheath).

experiment no.	temperature °C	nerve radius (r_0) mm	action potential amplitude in Ringer's mV	hydrochloride concentration (C_0) mM	pH	base concentration (C_B) mM	$t_{0.5}$ sec	$\frac{t_{0.5}}{r_0^2}$ sec/cm² ×10⁴	$t_{0.01}$ sec	$\frac{t_{0.01}}{r_0^2}$ sec/cm² ×10⁵
133	22.0	0.33	0.6	1	7.00	0.1	370	34.0	—	—
125	29.4	0.33	2.2	2	7.00	0.2	99	9.1	885	8.1
189	21.8	0.37	1.5	5	6.75	0.3	140	10.2	550	4.0
127	22.7	0.39	4.1	20	6.92	1.6	17	1.1	172	1.1
193	25.3	0.31	2.4	20	6.97	1.8	6	0.7	53	0.6
194	21.9	0.32	1.8	20	6.97	1.8	20	2.0	117	1.1
196	23.6	0.36	3.1	20	7.00	1.9	17	1.3	148	1.1
192	24.8	0.31	3.7	20	7.00	1.9	17	1.8	133	1.4
168	24.3	0.37	2.7	20	7.02	2.0	14	1.0	110	0.8
177	25.1	0.45	4.5	20	7.10	2.3	14	0.7	83	0.4
173	25.1	0.46	3.1	20	7.10	2.3	14	0.7	119	0.6
175	23.9	0.40	2.0	40	7.16	5.0	12	0.8	90	0.6
166	23.1	0.40	2.7	40	7.22	5.8	31	1.9	202	1.3
169	25.7	0.39	4.1	40	7.40	8.1	17	1.1	162	1.1
176	24.6	0.42	3.8	20	7.85	8.5	21	1.2	168	1.0
198	24.1	0.34	2.8	40	7.50	9.3	12	1.0	120	1.0
167	23.4	0.40	3.5	40	7.80	15.5	24	1.5	159	1.0

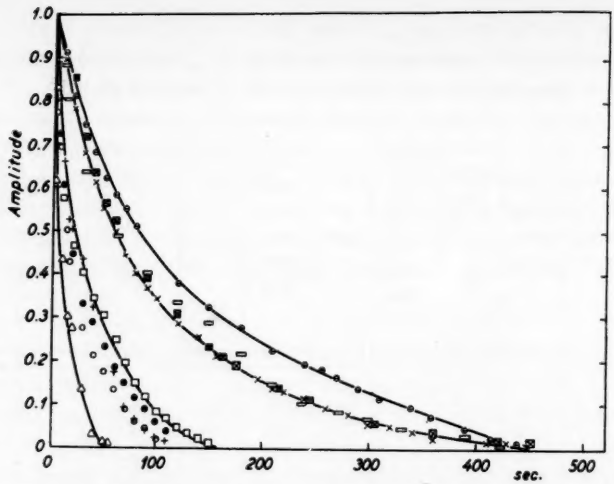


Fig. 23. Reduction in action potential amplitude during xylocaine anesthesia in nerves with and without sheath (temperature range: 21.5–25.3°C, see footnote p. 62). Base concentrations 1.8–2 mM in 5 nerves without (lower curves) and 4 nerves with sheath (upper curves).
Ordinate: amplitude in units of the amplitude in Ringer's.
Abscissa: seconds after application of the anesthetic.

Nerves without sheath:

□ Experiment 196: C_B = 1.9 mM, r_0 0.36 mm, pH 7.0
△ Experiment 193: C_B = 1.8 mM, r_0 0.31 mm, pH 6.97
● Experiment 192: C_B = 1.9 mM, r_0 0.31 mm, pH 7.0
○ Experiment 168: C_B = 2.0 mM, r_0 0.37 mm, pH 7.02
+ Experiment 194: C_B = 1.8 mM, r_0 0.32 mm, pH 6.97

Nerves with sheath:

▭ Experiment 154: C_B = 2.0 mM, r_0 0.40 mm, pH 7.02
⊖ Experiment 96: C_B = 1.8 mM, r_0 0.38 mm, pH 6.97
× Experiment 103: C_B = 1.8 mM, r_0 0.36 mm, pH 7.01
⊠ Experiment 128: C_B = 1.8 mM, r_0 0.42 mm, pH 6.99

Table 10.

Time to half action potential amplitude ($t_{0.5}$), to 20 per cent amplitude ($t_{0.2}$) and to block ($t_{0.01}$) in nerves with and without sheath (xylocaine).

	experiment no.	base concentration (C_B) mM	nerve radius (r_0) mm	$t_{0.5}$ sec	ratio	$t_{0.2}$ sec	ratio	$t_{0.01}$ sec	ratio
+ sheath	132	0.05	0.39	720	2.0				
— sheath	133	0.1	0.33	370					
+ sheath	126	0.2	0.38	198	2.0	670	1.9	1452	1.6
— sheath	125	0.2	0.33	99		360		885	
+ sheath	90	0.3	0.43	240	1.7	660	2.1	1250	2.3
— sheath	189	0.3	0.37	140		310		550	
+ sheath	76	1.6	0.44	67	3.9	186	3.1	414	2.6
— sheath	127	1.6	0.39	17		60		172	
+ sheath	161	5.5	0.46	44	1.4	124	1.5	330	1.6
— sheath	166	5.8	0.40	31		85		202	
+ sheath	160	8.1	0.44	45	2.6	126	2.3	306	1.9
— sheath	169	8.1	0.39	17		55		162	
+ sheath	114	9.6	0.42	24	2.0	83	2.1	235	2.0
— sheath	198	9.3	0.34	12		39		120	
+ sheath	162	13.7	0.43	50	2.1	143	2.2	369	2.3
— sheath	167	15.5	0.40	24		65		159	
			mean value:		2.2		2.2		2.1

illustrated in Figure 23 which shows examples of the diminution in action potential amplitude in the experiments on nerves subjected to xylocaine base concentrations of 1.8–2.0 mM, 5 without sheath and 4 with sheath. There was a clear difference in the time course between the two groups. Comparing the times when the amplitude of the action potential was reduced to 50 per cent, 20 per cent and 1 per cent, nerves without sheath were anesthetized about 4 times more rapidly than nerves with sheath. In this comparison no correction was made for differences in nerve diameter. Comparing the time course of the anesthesia in nerves which had the same diameter before removal of the sheath a somewhat smaller difference was found (Table 10). The sheathless nerves were anesthetized about twice as rapidly as "matched" nerves with sheath.

There is no apparent explanation for the finding in these experiments of

a factor 2 between anesthetic times for nerves with and without sheath as compared with a factor 4 in nerves unmatched for diameters and at the same anesthetic concentration. It is possible that in some of the nerves in the "unmatched" experiments the removal of sheath caused a certain separation of the single fibers so that the anesthetic diffused between them more rapidly, although there was no apparent difference in technique.

Table 11.

Time to half action potential amplitude ($t_{0.5}$) and to block ($t_{0.01}$) during xylocaine anesthesia at different temperatures.

experiment no.	temperature °C	hydrochloride concentration (C_0) mM	pH	base concentration (C_B) mM	nerve radius (r_0) mm	$\frac{t_{0.5}}{r_0^2}$ sec/cm²	$\frac{t_{0.01}}{r_0^2}$ sec/cm²
118	3	20	7.20	2.9	0.38	1.0×10^5	6.1×10^5
119	4.5	20	7.18	2.8	0.36	6.1×10^4	4.1×10^5
120	7	20	7.25	3.2	0.35	6.4×10^4	4.0×10^5
127	9	20	7.09	2.3	0.37	4.3×10^4	3.3×10^5
121	15	20	7.08	2.3	0.35	5.1×10^4	3.2×10^5
109	20	40	6.83	2.6	0.39	3.3×10^4	2.2×10^5
159	21.3	40	6.90	3.0	0.44	3.5×10^4	2.6×10^5
104	21.6	20	7.23	3.1	0.38	3.7×10^4	2.8×10^5

The effect of temperature on the course of anesthesia.

In the concentration range 2.3–3.2 mM xylocaine base the time course of anesthesia in nerves with sheath was investigated at temperatures between 3 and 21°C (Table 11, Figure 24). Between 3 and 13°C the time to half amplitude and the time to 1 per cent amplitude diminished 50 per cent ($Q_{10} = 2$). Although each nerve was investigated at one temperature only, the acceleration of the anesthesia could not be explained by individual variations or by differences in base concentration. In the temperature range 13–21°C there was no significant change in the time course of the anesthesia. In some experiments the temperature was above 22°C (maximally 26°C*)). These were performed with base concentrations below 2.3 mM or above 3.2 mM, and the influence of temperature could therefore not be evaluated

*) In one exceptional experiment – no. 126 – the temperature was 28.5°C.

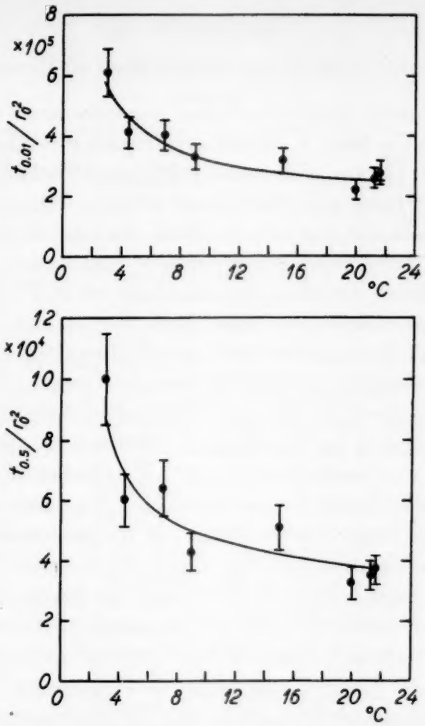


Fig. 24. Time to half the action potential amplitude ($t_{0.5}$) and to block ($t_{0.01}$) in xylocaine anesthesia as a function of temperature. Base concentration 2.3–3.2 mM.
Ordinate: t/r_0^2 where t is the time in seconds after application of the anesthetic and r_0 the nerve radius (sec/cm²).
Abscissa: temperature in centigrades.
The experiments were carried out with different nerves at the different temperatures. The standard deviation is indicated by the vertical lines.

directly. However, if $t_{0.5}$ and $t_{0.01}$ were corrected to a concentration range of 2–3 mM (Fig. 22) also in the experiments performed at 22–26°C the influence of differences in temperature was negligeable. These findings justify to pool all experiments performed at room temperature, within the temperature range 18–26°C.

DISCUSSION

Minimum concentration.

The minimum concentration of procaine was determined for the first time by GROS (1910) on the basis of clinical experiments carried out by HEINEKE and LÄVEN (1905). TREVAN and BOOCK (1927) used local anesthetics applied to the cornea of rabbits with the corneal reflex as indicator of anesthetic effect. BOEMINGHAUS and KOCHMANN (1929) determined the minimum concentration for procaine using a nerve-muscle preparation. To ensure that there was equilibrium between the anesthetic in and outside the nerve BOEMINGHAUS and KOCHMANN insist that the bathing time should be extended to 12–24 hours when low concentrations are used. It appears, however, from BOEMINGHAUS and KOCHMANN's own control experiments with a nerve in Ringer's for 24 hours, that such a long experimental time results in an increase in the threshold of the nerve to stimulation. With a higher threshold to stimulation it might be expected that the minimum concentration of the anesthetic would decrease. Therefore the experimental time should not be longer than necessary. In the experiments reported here the minimum concentration was determined which caused the action potential to disappear after 6–8 hours of exposure to the anesthetic. That this exposure time was considered sufficient to ensure equilibrium between the inner and outer solutions is based on the following considerations:

1) The minimum concentration found in experiments using 6–8 hours exposure time was lower or equal to that of other investigators both for xylocaine and procaine (see Table 12).

2) In the experiments concerned with the time course of anesthesia the lowest xylocaine base concentration able to block a nerve was 0.15 mM. Extrapolating the time required to reduce the action potential to one per cent of its original value to lower base concentration a minimum concentration was found of the same order as that determined directly after 6–8 hours (0.1 mM xylocaine base). At the minimum concentration $t_{0.01}$ is infinite, i.e. $1/t_{0.01} = 0$. The minimum concentration was therefore determined by extrapolating to the concentration at which $1/t_{0.01} = 0$.

The experiments concerned with the determination of the minimum concentration were carried out on nerves which were 25 per cent thinner than the nerves used in the investigation of the whole course of anesthesia. Therefore, the blocking time in these experiments was about 60 per cent of that in the experiments on the time course (maximally about $2\frac{1}{2}$ hours). Thus, using an experimental exposure time of 6–8 hours there should be a reasonable safety margin that equilibrium be reached between the inner and

Table 12.

Minimum concentrations for procaine and xylocaine determined by various authors.

author	year	anesthetic	method	type of nerve	pH	minimum concentration	
						hydrochloride	free base mM
HEINEKE & LÄVEN	1905	procaine	infiltration	sentitive human	?	0.1 %	
TREVAN & BOOCK	1927	procaine	cornea	sensitive rabbit	?		0.13
BOEMINGHAUS & KOCHMANN	1929	procaine	nerve-muscle preparation ***)	sensitive	7.0?	0.05 %	0.15 *)
				motor *Rana temporaria*	7.0?	0.075 %	0.23 *)
BENNETT et al.	1942	procaine	recording of action potential	mixed *Rana pipiens*	7.38	0.1–0.15 mM	0.02–0.03 *)
EHRENBERG	1948	procaine	nerve-muscle preparation **)	motor *Rana arvensis*	7.39		0.262
		xylocaine					0.815
SKOU	1954	procaine	nerve-muscle preparation	motor *Rana esculenta*	6.0	750–500 mg %	0.024–0.016 *)
					7.0	150–100 mg %	0.047–0.032 *)
					7.35	80–70 mg %	0.058–0.050 *)
					8.0	40–30 mg %	0.09 –0.12 *)
RUD	1958	procaine	recording of action potential	mixed *Rana esculenta*	6.12		0.013 *)
					7.08		0.021 *)
					7.65		0.026 *)
		xylocaine			6.00		0.04 *)
					7.10		0.11 *)

*) base concentration calculated from equation (4) p. 27, $f_{BH^+} = 0.73$.

**) minimum concentration calculated from diffusion theory.

***) values from 12 hour experiments.

outer fluids even at concentrations only a few per cent above the minimum concentration.

Other authors have sought to avoid a long experimental time in determining the minimum concentration. SKOU (1954) removes the nerve sheath and separates the nerve into many fine threads so that an equilibrium between the inner and outer fluids is attained more rapidly. EHRENBERG (1948) used high concentrations and calculated the minimum concentration for procaine and xylocaine on the basis of the theory of free diffusion into the nerve. BENNETT et al. (1942), who used the decrease in amplitude of the action potential as measure of anesthetic effect, determined the minimum concentration as that concentration at which the amplitude was reduced at least 10 per cent and not more than 50 per cent within one hour. They found the same minimum concentration for procaine as that found in these studies. SKOU (1954) used the disappearance of a muscle contraction as measure of anesthetic effect in a nerve-muscle preparation and found a minimum concentration for procaine which was double the minimum concentration found in these studies at pH 6 and three times the present value at pH 7.3–8. SKOU (personal communication) suggests that the difference between the two studies may be that he uses a phosphate buffer whereas in this study a combination of phosphate and bicarbonate buffer was used.

It might furthermore be considered that SKOU records solely the response of the motor fibers, whereas the action potential in the study presented here is recorded from all fibers in the A group. The motor fibers belong to the α type of nerve fibers in the A group and are characterized by large diameter. The action potential contains also contributions from potentials from other A fibers (β, γ and δ), of smaller diameter than the α fibers. It is reasonable to suppose that there may be a spectrum of minimum concentrations corresponding to the various types of fiber. With the disappearance of the action potential as measure of anesthetic effect the minimum concentration is that of those fibers with the highest minimum concentration. These are probably α fibers corresponding to GASSER's (1943) assumption that the blocking time is proportional with fiber diameter. In this case the minimum concentration was determined for the same fibers in SKOU's as in the present studies.

The experiments contribute no information as to the breadth of such a spectrum of minimum concentrations if such a spectrum exists. To determine the minimum concentration in a whole nerve for the most sensitive fibers, one should in principle find the smallest concentration which just serves to diminish the action potential amplitude. This procedure is however not practicable since as mentioned on page 93 concentrations under the minimum concentration reduce the action potential amplitude of the single nerve fibers.

Base concentration versus acid concentration as anesthetic agent.

Comparing the time course of anesthesia in experiments at the same base concentration but with different concentrations of acid, there was no ten-

dency for solutions with high acid concentration to have a more rapid anesthetic effect. Comparison of experiments with different concentrations of the base component revealed that the anesthesia progressed more rapidly as the concentration of base increased. From this fact it is concluded that the course of anesthesia is for the most part determined by the concentration of base. This is in agreement with GROS (1910), TREVAN and BOOCK (1927), GARDNER, SEMB and GRAHAM (1934) and EHRENBERG (1948).

The fact that only the free base and not the acid is soluble in lipids (LÖFGREN 1948, SKOU 1954) may account for the anesthetic action of the base component, since it can be bound to the lipids of the nerve fiber membrane.

The dependency of the minimum concentration on pH.

TREVAN and BOOCK (1927) and SKOU (1954) found that the minimum concentration of procaine base was lower at low than at high pH. The same was observed in the present experiments both for procaine and xylocaine. In the pH range 6–7 the same slope of pH effect for procaine was found as seen by SKOU (1954), but in the range of pH 7–8 a somewhat greater effect was seen (see Table 12).

That the minimum concentration varies with pH might be explained in various ways:

1) TREVAN and BOOCK (1927) assume that the buffer capacity of the nerve tissue causes the pH of the anesthetic solution within the nerve to approach that of the nerve. Thus with acid solutions the base concentration in the nerves should increase. The anesthetic effect would be more marked than expected from the outer base concentration. The reverse should obtain for anesthetic solutions at a pH higher than that of nerve tissue. SKOU's experiments indicate however that the buffer capacity of the nerve is not the only explanation after all. His experiments were carried out on a teased-out nerve and the pH was maintained constant by changing the anesthetic solution frequently. With this experimental technique it is unlikely that the nerve has a sufficiently large buffer capacity to change the pH. In the experiments reported here with intact nerves, an attempt was made to ensure that the nerve had the same pH as the anesthetic by immersing it in Ringer's solution at the pH to be used for an hour before the experiment. Since the minimum concentrations found show approximately the same pH dependency as found by SKOU (1954) it may be concluded that the effect of the buffer capacity of the nerve tissue is of little importance.

2) Since the protolysis of the anesthetic is pH dependent, a higher concentration of the acid component is required at low than at high pH to achieve

the same base concentration. It might therefore be argued that the acceleration of the anesthetic effect with decreasing pH for the same base concentration in the region of small concentrations must be due to the fact the acid component also has anesthetic properties. This problem was discussed on page 69 where it was demonstrated that if the acid component has an anesthetic effect it must be considerably less than that of the base component.

3) A third possible explanation for the fact that the minimum concentration of the base decreases with decreasing pH would be a pH dependence of the susceptibility of the nerve to anesthesia. A decrease in pH is associated with an increased threshold to stimulation (ADRIAN 1920, LEHMANN 1937 a). Such an increase in threshold in an anesthetized nerve, in which the membrane current is reduced and is barely sufficient to stimulate the adjacent section of the nerve, would be expected to cause a block at a lower concentration of the anesthetic.

That the susceptibility of the nerve to local anesthetic is pH dependent might also be explained on the basis of the mechanism by which local anesthetics are assumed to block the nerve impulse. It appears likely that the local anesthetic blocks the nerve fiber by reducing the permeability of its membrane to sodium (see p. 13). Permeability to sodium is likewise reduced when calcium ions are bound to the membrane, that is, calcium acts synergistically with local anesthetics (see page 14). The assumption has been made, that calcium is bound more firmly to the nerve membrane in an acid than in an alkaline medium (DETTBARN and STÄMPFLI 1957). A given concentration of calcium and local anesthetic should therefore be most effective at low pH and the minimum concentration would be expected to be lower at low pH.

4) An explanation for the fact that the minimum concentration increases with increasing pH might be that a certain concentration of anesthetic must be bound to the nerve membrane in order to block it. SKOU (1954) found that a local anesthetic is bound to nerve tissue partially by adsorption and partially by solution of the base component in the dry phase of the nerve chiefly in the lipids. The partition coefficient of the base component of procaine between the dry and fluid phases of nerve diminished with increasing pH (SKOU 1954). Therefore, to obtain that a given amount of base be bound to the dry phase of nerve demands more base at high than at low pH.

The time course of anesthesia.

With the amplitude of the action potential as measure it was possible to follow the time course of anesthesia from the application of the anesthetic to full nerve block.

Anesthesia advanced rapidly at first and then more slowly. For the lowest **xylocaine** base concentrations investigated (0.05–0.16 mM) it took on the average about 10 minutes for the amplitude to fall to half and for 0.15–0.16 mM solutions about 70 minutes to achieve full block. With increasing concentrations of xylocaine base the nerve was anesthetized more rapidly. This was especially marked in the range of concentrations up to about 1 mM xylocaine base. In the concentration range 1.0–1.5 mM the time to half amplitude was about 1 minute and the blocking time about 8 minutes. For the higher xylocaine base concentrations investigated (8.7–13.7 mM) the time to half amplitude was on the average about one half minute and the time to full block about 4 minutes. Thus, whereas the course of anesthesia progressed about 10 times more rapidly at a concentration 10 times above the lowest concentration, it was only about twice as rapid with a 10 times increase of large concentrations.

In the case of **procaine** there were not sufficient experiments to allow a detailed description of the couse of anesthesia as a function of concentration. The tendency is, however, the same as that described above for xylocaine (Fig. 21).

Comparison of the time course of anesthesia with xylocaine and procaine.

Since most experiments with procaine were performed with 1 mM procaine base, the course of anesthesia will be compared with xylocaine at the same concentration. The time to half amplitude was about 60 per cent and the time to block about 40 per cent longer for xylocaine than for procaine (Figure 20 and Table 6). It may be questioned whether the more rapid anesthetic effect of procaine than of xylocaine at the same base concentration may be related to the fact that procaine has a smaller minimum concentration. When the base concentration is expressed in units of the minimum concentration ($C_B/C_m = 40$) the time to half amplitude was twice as long for procaine as for xylocaine and the blocking time was one and a half times as long (Figure 22 A, B and Table 6). In other words, xylocaine acts more rapidly than procaine when the substances are compared at base concentrations which are the same multiple of the minimum concentration. The time course of anesthesia can therefore not be explained solely in terms of the concentration of the active anesthetic component (base concentration) or the minimum concentration, but must depend on other factors as well as discussed in chapter 5.

Ehrenberg (1948) investigated the time to block for xylocaine and procaine using a nerve-muscle preparation. The xylocaine experiments were

carried out with base concentrations of from 0.85 to 1.8 mM (pH 7.39). Corresponding blocking times varied from 3000 to 700 seconds (with the exception of one extreme value of 5640 seconds with $C_B = 0.89$ mM). In the experiments described here (Figure 20) the blocking times in the same concentration range varied from about 900 to 400 seconds. In the case of procaine, the smallest base concentration (0.27 mM) used by EHRENBERG (1948) gave a blocking time of 3840 seconds and the highest concentration (0.63 mM) gave a blocking time of 660 seconds. Compared with the experiments in my study (Figure 21) blocking times of 700 and 400 seconds would be expected.

There is no clear explanation for the difference in blocking times in EHRENBERG's and in my experiments. The only difference in technique which might be of importance is that EHRENBERG did not use carbon dioxide bicarbonate buffer whereby the minimum concentration might be increased (SKOU, personal communication). It does not however seem likely that this can be the whole explanation for the difference in blocking times.

The importance of the sheath for the time course of anesthesia.

The importance of the sheath for the time course of anesthesia has often been investigated by comparing the anesthesia of nerves with and without sheath. I have found that a nerve stripped of its sheath is blocked about 2–4 times as rapidly by xylocaine as a nerve with intact sheath. This is in agreement with TASAKI (1953) who found that removal of the sheath reduced the blocking time to half or less (urethane). In a short abstract TRUANT and LANZONI (1952) described that xylocaine, procaine and other local anesthetics blocked the action potential 8–10 times more rapidly when the sheath was removed. In other investigations of local anesthetic action on nerves with and without sheath the experimental conditions were quite different. SKOU (1954) investigated the effect of various local anesthetics on nerves in a nerve-muscle preparation using muscle contraction to indicate passage of the nerve impulse. By removing the sheath and separating the nerve into many fine threads, the time for blocking of the motor fibers was diminished about 10 times as compared with intact nerves. This difference is not surprising since it is known that local anesthetics applied to single nerve fibers block conduction almost instantaneously (KATO 1936, TASAKI and TAKEUCHI 1942 and TASAKI 1953). FENG and LIU (1949) found a still greater difference at 2.5 and 4°C in that cocaine blocked the nerve action potential 35–50 times faster without than with sheath.

The value of experiments carried out on sheathless nerves has been much

discussed in the literature. LORENTE DE NÓ (1950) doubts the value of such experiments, because in his opinion removal of the sheath damages the fibers and alters the characteristics. He concludes that it has not been shown that the nerve sheath itself delays anesthesia. Against LORENTE DE NÓ's arguments it may be said that he himself (1950) found that nerve roots lacking an epineurium and having only an endoneurium were blocked about 10 times more rapidly by a sodium-free solution than a nerve with sheath. NORDQUIST (1952 b) instead of dissecting the sheath away subjected the nerve to the action of hyaluronidase which dissolves the connective tissue so diffusion through it occurs more easily. Applying procaine to a nerve treated in this way, he found that the blocking time was diminished to about half. In the case of nerves without sheath hyaluronidase had no effect on the action of procaine. CRESCITELLI (1951) reported a more rapid action of antihistamine on nerves without sheath. By washing the substances out of the nerve again, by "rolling" the sheath back in place and repeating the experiment with sheath he found that the blocking time again approached the values in the intact nerve. He concluded therefore that removal of the sheath does not alter the characteristics of the fibers (see also p. 94).

Chapter 5

COMPARISON OF THE EXPERIMENTAL TIME COURSE OF ANESTHESIA WITH THAT PREDICTED FROM FREE DIFFUSION OF THE ANESTHETIC INTO THE NERVE

The time course of anesthesia in a whole nerve was found to differ from that of a single nerve fiber in which block occurs instantaneously. Hence the time course of anesthesia in a whole nerve is determined at any rate in part by diffusion into the nerve. This was assumed in earlier investigations by Bennett et al. (1942) and by Ehrenberg (1948) who, by use of diffusion theory, calculated the minimum concentration and the diffusion coefficient for the anesthetic into a nerve.

In this chapter a more detailed discussion will be given of the extent to which it is possible to describe the time course of xylocaine anesthesia in a peripheral nerve on the basis of the theory of free diffusion. Obviously in a whole nerve there is nothing resembling free diffusion in a homogeneous medium. In the first place the nerve is surrounded by a sheath; in the second place the anesthetic can only penetrate the interfibrillar spaces of the nerve; finally the diffusion process is complicated by the fact that the anesthetic is bound to the nerve tissue as it penetrates into the nerve and this implies that one is not dealing with a diffusion process which can be described by using the simple Fick law of diffusion. However, under certain conditions diffusion can progress qualitatively in the same way as free diffusion even if the diffusing substance undergoes a physical or chemical reaction in the course of its diffusion (see page 99). In this case diffusion would progress more slowly, as would be evidenced by a smaller diffusion coefficient than in free diffusion. The inhomogeneity of the nerve would likewise delay diffusion, the area through which the substance diffuses into the nerve being less than the total surface area of the nerve. To differentiate the diffusion coefficient in a homogenous medium from that in an inhomogenous medium, the latter can be described as the "effective diffusion coefficient" (Shanes and Berman 1955). In the discussion below, the diffusion coefficient in nerve is used in the sence of *apparent* or *effective* diffusion coefficient.

EHRENBERG's (1948) calculation of the minimum concentration and diffusion coefficient from diffusion theory are based solely on the blocking time. The premise for this procedure is that the time course of anesthesia can be described from the theory of free diffusion, i.e. that the factors which complicate diffusion in a nerve do not invalidate the applicability of the theory of free diffusion to account for the course of anesthesia. EHRENBERG has not stated to what extent this premise has been fulfilled and it is uncertain to what extent it is justified to determine minimum concentration and diffusion coefficient from the blocking time alone. The material presented in my study, where the time course of anesthesia was determined at various concentrations and where a separate experimental determination of the minimum concentration was carried out, should be suitable to investigate to what extent a *simple diffusion concept* can account for the diminution in action potential amplitude and whether the minimum concentration calculated on this basis is identical with that determined experimentally.

The mathematical basis for calculation of diffusion of the anesthetic into nerve assuming free diffusion.

Let the nerve at time zero be placed in a large volume of a solution of the local anesthetic at concentration C_e. Considering the nerve as an infinitely long homogeneous cylinder, the diffusion of the anesthetic into and within the nerve will be determined by the following equation:

$$\frac{\partial^2 C}{\partial r^2} + \frac{1}{r}\frac{\partial C}{\partial r} = \frac{1}{D}\frac{\partial C}{\partial t};\ 0 \leqq r \leqq r_0\ \ t \geqq 0 \qquad (1)$$

with the boundary conditions:

$$C = 0 \text{ for } t = 0 \text{ and } 0 \leqq r < r_0$$

and

$$C = C_e \text{ for } r = r_0 \text{ and } t \geqq 0.$$

$C = C(r, t)$ indicates the concentration of anesthetic at a distance r from the nerve axis at time t, whereas D is the diffusion coefficient of the anesthetic in the nerve and r_0 is the radius of the nerve.

Solving equation (1) the following expression for the variation of the concentration of anesthetic through the nerve is obtained (compare for instance CARSLAW and JAEGER 1947):

$$C(r, t) = C_e \left\{ 1 - 2 \sum_{n=1}^{\infty} e^{-\frac{D\beta_n^2 t}{r_0^2}} \frac{J_0\left(\frac{r\beta_n}{r_0}\right)}{\beta_n J_1(\beta_n)} \right\}, \qquad (2)$$

where J_0 and J_1 are Bessel functions and β_n's are the roots $J_0(\beta_n) = 0$.

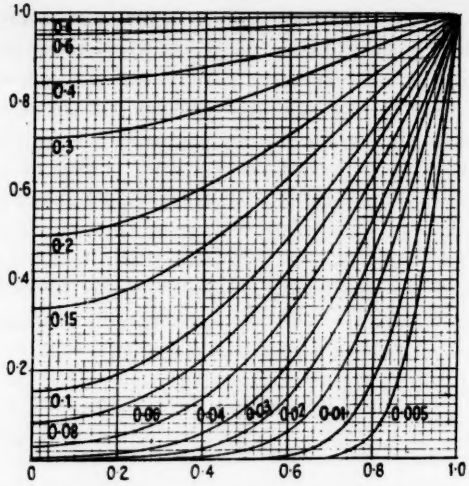


Fig. 25. The concentration distribution in a cylinder at various times after its placement in a medium containing a diffusible substance of constant concentration (C_e). The initial concentration in the cylinder is zero. The different curves give the concentration within the cylinder as a function of the distance from the axis at different times indicated on the curves in terms of Dt/r_0^2, where D is the diffusion coefficient (CARSLAW and JAEGER 1947). Ordinate: concentration in units of the outer concentration (C_e). Abscissa: distance from the axis in units of the radius (r_0).

In Figure 25 these distributions of concentrations at various times are demonstrated graphically.

It follows from expression (2) that the time required to obtain a certain fraction of the outer concentration C_e at distance r from the axis of the nerve is inversely proportional with the diffusion coefficient D of the anesthetic and proportional with the square of the nerve radius r_0. Hence, for various anesthetics the course of anesthesia will be the faster the greater the diffusion coefficient in the nerve.

From the distribution of concentrations as given by (2) and Figure 25 and with the assumptions that there exists a minimum concentration which is the same for all nerve fibers in the A group and that the action potential amplitude is proportional with that area in the nerve where the concentration is less than the minimum concentration, the time course of the diminution in amplitude can be predicted. In Figure 26 A this is shown for different values of the ratio between the outer concentration C_e and the minimum concentration C_m, the time being expressed in arbitrary units as Dt/r_0^2. It is seen that the diminution in action potential amplitude occurs more

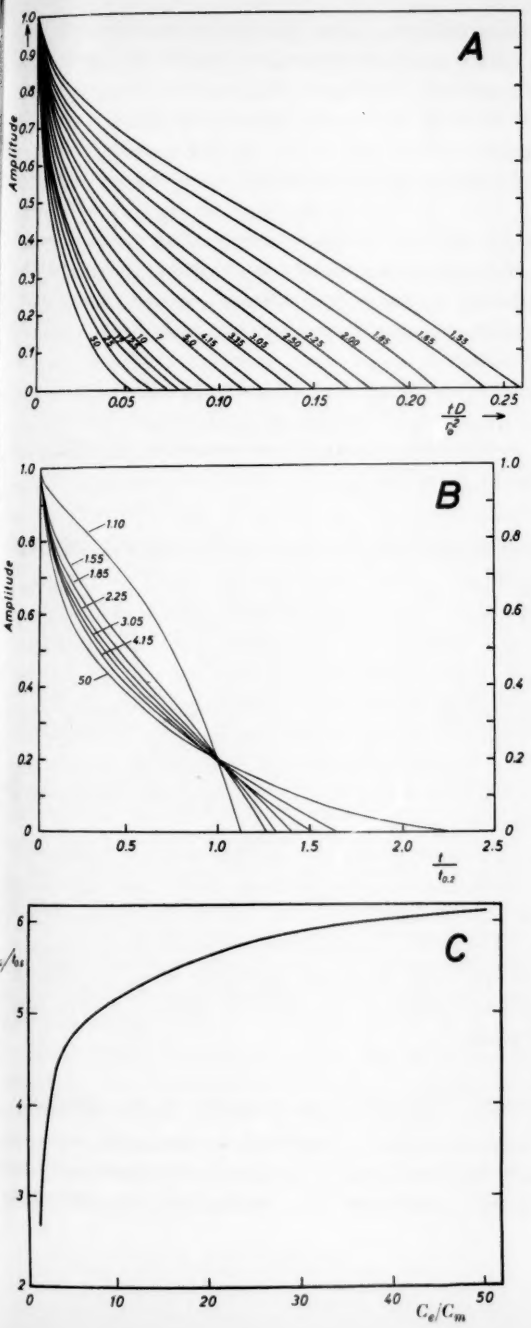


Fig. 26. A: The theoretical time course of anesthesia in a nerve with various outer concentrations (C_e). The figures on the curves give the concentration in units of the minimum concentration (C_m). (Constructed from Fig. 25).

Ordinate: calculated action potential amplitude in units of the initial value.

Abscisse: time in terms of Dt/r_0^2 where D is the diffusion coefficient, t time in seconds and r_0 nerve radius in centimeters.

B. To compare the relative time course of anesthesia with various outer concentrations the calculated curves from (*A*) have been normalized to intersect at amplitude 0.2.

Ordinate: calculated action potential amplitude in units of the initial value.

Abscisse: time after application of the local anesthetics in units of the time required to reduce the amplitude to 0.2.

C: The ratio $t_{0.2}/t_{0.6}$ (ordinate) for the calculated time courses of anesthesia as a function of the outer concentration (C_e) in units of the minimum concentration (abscissa). $t_{0.2}$ and $t_{0.6}$ are the times required to reduce the action potential amplitude to 20 and 60 per cent of its initial value, respectively.

rapidly as the outer concentration C_e of the anesthetic increases. To illustrate the effect of the outer concentration on the shape of the curves the relative time courses are compared in Figure 26 B in that all curves have been normalized to pass through the same point of amplitude 0.2. The relative time course depends on the ratio C_e/C_m in that the last phase of anesthesia occurs relatively most rapidly when the outer concentration of the anesthetic is small.

The criteria to determine whether the experimental time course of anesthesia with different concentrations and in nerves of various thickness can be described in mathematical terms of free diffusion are therefore that:

1) there is agreement between experimental and theoretical time courses in individual experiments;
2) the calculated minimum concentrations are identical with those found experimentally;
3) the same diffusion coefficient is obtained in experiments with different concentrations of the local anesthetic.

The calculated time course of anesthesia as compared with the experimental.

To compare the theoretical and experimental time course of anesthesia the theoretical curve was selected from Figure 26 A whose form was similar to the experimental. This was found as the theoretical curve which had the same ratio between times to 20 per cent ($t_{0.2}$) and 60 per cent ($t_{0.6}$) of the initial action potential amplitude as the experimental curve (Fig. 26 C). To test the agreement between this curve and the course of the experimental curve, the time axis of the theoretical curve was adjusted by determining D in the expression Dt/r_0^2 in such a way that the best possible agreement is obtained between the two time courses.

In this way it was found possible in the case of experiments with *xylocaine* over the whole range of concentrations used to obtain such a close agreement between theoretical and experimental anesthetic course that the maximal deviation did not exceed 5 per cent of the initial amplitude in the range of amplitude from 100 to 15 per cent. Below an amplitude of 15 per cent the experimental diminution in amplitude occurred in general slower than calculated from free diffusion.

The minimum concentration calculated from the theory of free diffusion.

The theoretical course of anesthesia in Figure 26 A selected according to the ratio $t_{0.2}/t_{0.6}$ similar to the experimental course was characterized by the ratio between C_B ($= C_e$) and C_m. Since the base concentration C_B used in the

experiments was known, C_m, the minimum concentration, could be calculated. With such a determination of the minimum concentration both the theory and the experimental error in the determination of $t_{0.2}/t_{0.6}$ limited the range of concentrations at which the determination could be carried out with reasonable certainty. As regards the theory, it is apparent from Figure 26 B that the relative time course varied less with the outer concentration as this became progressively larger:

An increase in outer concentration from 1.5 to 3 times the minimum concentration was accompanied by a greater change in the relative time course than an increase in concentration from 3 to 50 times the minimum concentration. Furthermore, in the range of concentration 4–50 times the minimum concentration, the relative time course varied only a few per cent in the amplitude range 100–20 per cent. Thus, on theoretical grounds alone the relative time course was less suitable to determine the minimum concentration as the outer concentration increased (Fig. 26 C). The experimental error in the determination of the ratio $t_{0.2}/t_{0.6}$ increased similarly with increasing outer concentrations of anesthetic. With a concentration about two times the minimum concentration the error was about $\pm$ 1 per cent, with 10 times the minimum concentration it was $\pm$ 2 per cent and with 50 times the minimum concentration it was $\pm$ 5 per cent. With these experimental errors the uncertainty in the determination of the minimum concentration was according to the theoretical correlation between C_B/C_m and $t_{0.2}/t_{0.6}$ (Fig. 26 C) $\pm$ 2 per cent with an outer concentration two times the minimum concentration, $\pm$ 15 per cent at 10 times the minimum concentration and $\pm$ 60 per cent with 50 times the minimum concentration. A determination of the minimum concentration from the experimental time course of anesthesia was therefore only possible with reasonable certainty by applying concentrations less than about 10 times the minimum concentration.

The calculated and the experimentally determined minimum concentrations for the 46 experiments with xylocaine on whole nerve are presented in Table 13. With low outer concentrations the calculated minimum concentration was equal to or less than that found experimentally and with increasing outer concentrations the calculated values were greater than those found experimentally.

In the range of outer concentrations up to four times the minimum concentration the experimentally and theoretically determined minimum concentrations agreed to within 30 per cent in 7 of 10 experiments and in the remaining three experiments (outer concentration less than 2 C_m) the calculated minimum concentration was 3–5 times less than the experimental. In 36 experiments with outer concentrations of more than four times the

Table 13.

Experimental and calculated minimum concentration and diffusion coefficient for xylocaine.

experiment no.	base concentration C_B mM	pH	experimental minimum concentration $C_{m(exp.)}$ *) mM	$\frac{C_B}{C_{m(exp.)}}$	calculated minimum concentration $C_{m(calc.)}$ mM	diffusion coefficient cm²/sec ×10⁻⁷	$\frac{C_{m(calc.)}}{C_{m(exp.)}}$
156	0.15	6.88	0.10	1.5	0.10	1.4	1.0
115	0.15	6.88	0.10	1.5	0.02	1.0	0.2
123	0.16	6.90	0.10	1.6	0.08	1.7	0.8
124	0.20	7.00	0.105	1.9	0.028	1.0	0.3
126	0.20	7.02	0.105	1.9	0.02	1.0	0.2
116	0.24	7.09	0.11	2.2	0.10	1.5	0.9
90	0.30	6.77	0.09	3.3	0.117	3.2	1.3
92	0.33	6.84	0.094	3.5	0.10	3.7	1.1
136	0.33	6.84	0.094	3.5	0.10	3.3	1.0
91	0.41	6.92	0.10	4.1	0.09	3.4	0.9
93	0.48	6.98	0.103	4.7	0.26	7.5	2.5
106	0.48	6.38	0.065	7.4	0.28	12.2	4.3
107	0.63	6.50	0.072	8.75	0.29	7.8	4.0
86	0.63	7.11	0.11	5.7	0.30	7.8	2.7
94	0.85	7.28	0.122	7.0	0.36	5.2	3.0
84	0.87	7.29	0.122	7.1	0.31	4.3	2.5
99	0.96	6.70	0.085	11.3	0.21	3.0	2.4
157	1.0	6.70	0.085	11.75	0.40	7.6	4.7
158	1.0	6.70	0.085	11.8	0.45	12.9	5.3
87	1.4	7.54	0.14	10.0	0.16	2.7	1.1
88	1.4	7.55	0.14	10.0	0.21	3.3	1.5
100	1.5	6.89	0.097	15.4	0.78	12.6	8.0
101	1.59	6.92	0.10	15.9	0.32	2.3	3.2
76	1.62	6.93	0.10	16.2	0.57	10.0	5.7
95	1.74	6.95	0.10	17.4	1.08	18.0	10.8
96	1.78	6.97	0.10	17.8	0.60	6.1	6.0
103	1.8	7.01	0.105	17.2	0.69	9.0	6.6
128	1.8	6.99	0.105	17.2	0.27	6.3	2.6
154	2.0	7.02	0.105	19.1	0.50	6.7	4.8
109	2.63	6.83	0.093	28.2	1.15	14.8	12.4
159	3.0	6.90	0.10	30.0	0.40	4.9	4.0
104	3.1	7.23	0.12	25.8	1.24	11.4	10.3
79	3.47	7.30	0.123	28.2	0.07	2.3	0.6
108	4.36	7.44	0.132	33.6	0.17	3.9	1.3
130	4.47	7.09	0.11	40.6	0.60	7.3	5.5
110	4.78	7.12	0.112	42.7	0.24	4.0	2.1
111	5.13	7.16	0.115	46.7	2.0	12.4	17.0
161	5.5	7.57	0.14	39.3	3.40	40.0	24.0
98	6.3	7.66	0.147	42.8	0.38	3.9	2.6
97	6.31	7.64	0.146	43.2	0.79	5.8	5.4
160	8.0	7.40	0.13	61.6	3.60	20.6	27.7
112	8.71	7.44	0.132	67.0	0.60	7.1	4.5
114	9.55	7.49	0.136	70.8	2.58	16.2	19.0
113	10.0	7.52	0.138	72.4	2.80	23.5	20.0
162	13.7	7.72	0.152	90.0	4.70	12.5	31.0
(102	0.19	5.67	0.018	10.5	0.002	0.7	0.1)

*) Values from Fig. 18.

minimum concentration, the minimum concentrations calculated from diffusion theory were on the average eight times greater than the directly determined values (extreme value 1.1 and 31 times).

The fact that the error in the calculated values is relatively greater when the concentration is high can explain a wide variability with high outer concentrations. It can however not explain the systematic difference between the calculated and the directly determined minimum concentrations with small and large concentrations nor can it explain the wide variability with outer concentrations as low as four times the minimum concentration. The feasibility of determining the minimum concentration from the time course of anesthesia with outer concentrations up to four times the minimum concentration is uncertain since there were only 10 experiments in this range.

The diffusion coefficient calculated simultaneously with the minimum concentration from the theory of free diffusion.

The time axis for the theoretical decay in action potential amplitude which had a shape similar to that of the experimental curve was adjusted by determining the value for the diffusion coefficient which gave the best agreement between experimental and theoretical time course (cf. p. 88). The diffusion coefficients determined in this way for the 46 experiments with xylocaine on whole nerve are given in Table 13, column 7. With base concentrations of 1.5–4 times the minimum concentration the calculated diffusion coefficient varied in the 10 experiments from 1.0–3.7 $\times 10^{-7}$ cm²/sec. With base concentrations of 4–90 times the minimum concentration the calculated diffusion coefficient averaged 8.7 $\times 10^{-7}$ cm²/sec with a variation from 2 to 23 $\times 10^{-7}$ cm²/sec. Two extreme values (0.7 and 40 $\times 10^{-7}$ cm²/sec) were not included in the calculation of mean value. That the diffusion coefficients corresponding to base concentrations greater than four times the minimum concentration showed such wide variability was a result of the widely varying calculated minimum concentrations and no real significance can therefore be attached to them.

Conclusion: As concerns the agreement between the experimental and the theoretical time course in experiments with *xylocaine*, agreement was obtained within the range of action potential amplitudes from 100 to 15 per cent of the initial value by suitable selection of minimum concentration and diffusion coefficient. With lower amplitudes the experimental time course was slower than the theoretical.

As concerns the comparison between the experimentally found and the calculated minimum concentrations there was no agreement in most experi-

ments. In addition to the fact that the calculated minimum concentrations varied widely there was a systematic disagreement in that the calculated minimum concentrations were higher as the outer concentrations increased.

Finally, as concerns the diffusion coefficient this varied in the various experiments by a factor of ten.

Hence the model of free diffusion of the anesthetic into the nerve cannot be used to calculate minimum concentrations which agree with the experimentally determined minimum concentrations and the diffusion coefficients in different experiments calculated therefrom are not identical.

FACTORS COMPLICATING THE DESCRIPTION OF THE TIME COURSE OF ANESTHESIA IN TERMS OF FREE DIFFUSION

The analysis of the mathematical expression describing free diffusion into the nerve indicated, that it should be possible from the time course of anesthesia with concentrations up to 10 times the minimum concentration to calculate an effective diffusion coefficient and minimum concentration, *if there were no other conditions in the nerve invalidating the use of this expression.* This seems, however, to be the case in that it was not possible in this range of concentrations to calculate minimum concentrations identical with those found experimentally. An attempt is made below to evaluate the complicating factors which could invalidate the use of the theory for free diffusion into nerve.

Action potential amplitude as a measure of the number of active nerve fibers.

Even if there is no direct proof it is usually assumed that the action potential amplitude is proportional to the number of active nerve fibers. This assumption was made use of, for example, in GASSER and ERLANGER's (1937) reconstruction of the action potential of a whole nerve by summation of the potentials of individual nerve fibers.

The effect of subminimal concentrations of anesthetic on action potential amplitude.

If it is assumed that the action potential amplitude is proportional with that area of the nerve in which the concentration of the local anesthetic is less than the minimum concentration, it must also be assumed that the minimum concentration has an all or none effect on action potential amplitude, such that concentrations above the minimum concentration block the action potential instantaneously, whereas subminimal concentrations have no effect on the amplitude. In fact TASAKI and TAKEUCHI (1942) found that

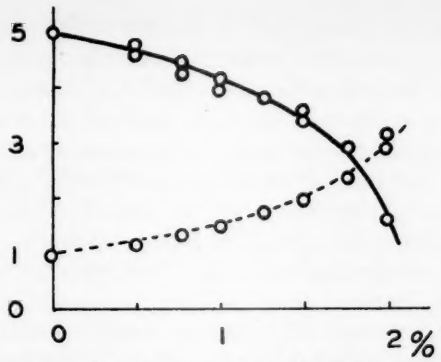


Fig. 27. The effect of subminimal concentrations of urethane on a single node in a frog nerve fiber (TASAKI and TAKEUCHI 1942). The action current (upper curve) and the threshold of stimulation (lower curve) as a function of concentration.
Ordinate: threshold in units of the threshold in normal nerve. The normal size of action current is taken as being equivalent to five times the normal threshold.
Abscissa: the concentration of urethane in per cent.

urethane in subminimal concentrations diminished the action potential amplitude and conduction velocity and increased the threshold to stimulation of single nerve fibers (Figure 27). Subminimal concentrations of cocaine, which should be more comparable with xylocaine and procaine than urethane, exert the same effect although the effective concentration range is considerably less for cocaine than for urethane (TASAKI 1958).

The effect of subminimal concentrations of xylocaine and procaine on the time course of anesthesia could only be estimated roughly, but the decrease in amplitude occurred more rapidly in the beginning than was calculated whereas there was no effect on blocking time. The relative time course at the end of anesthesia should therefore be slower than to be expected from the theoretical curves of Fig. 26 A. This effect would be greatest with the smallest concentrations.

The nerve considered as an homogeneous circular cylinder.

Measurements of the diameter of nerve demonstrated that at the points subjected to the anesthetic it may be considered circular in cross section. Furthermore, no differences in nerve diameter were found within the 10 mm exposed to the anesthetic nearest to the nerve's peripheral end, i.e. where the nerve was thinnest.

On the other hand the nerve is not homogeneous since there is a nerve sheath and nerve fibers surrounded by interstitial fluid.

The nerve sheath as diffusion barrier.

The importance of the nerve sheath as diffusion barrier has been a subject for disagreement. SHANES (1954) attempted to demonstrate the role of the sheath directly by filling stripped off sheath with a solution containing radioactive sodium and closing the ends. For comparison a whole nerve was saturated with radioactive sodium and thereafter the diffusion was measured as it occurred into a Ringer's bath in both cases. The time for diffusion of sodium was practically the same through the sheath alone as through the intact nerve, and SHANES concludes that it is the sheath alone which constitutes the diffusion barrier.

According to LORENTE DE NÓ (1950) the sheath in itself presents no resistance to diffusion. Its effect is that it packs nerve fibers and endoneurium so closely together that penetration is rendered difficult. This assumption is supported by experiments which indicate that sheathless nerves placed for 2–4 hours in Ringer's solution increase in weight in contrast to intact nerves which do not increase in weight. According to FENG and LIU (1949) this increase is 10 per cent and according to SHANES (1953) it may amount to up to 40 per cent. Correspondingly, TRUANT and LANZONI (1952) and FRANKENHAEUSER and NYSTRÖM (1954) found that nerves without sheath increase in diameter when placed in Ringer's solution. In my experiments the increase in diameter averaged 6 per cent in the course of one hour. NYSTRÖM and SÖDERBERG (1955) found that the increase in diameter depends on the hydrogen ion concentration and is maximal at pH 5.

The findings described on page 73 indicate that the sheath has a delaying action on diffusion even if the results from experiments on nerves without sheath might be affected by changes in the nerve due to removal of the sheath.

In the xylocaine experiments on nerves *with* sheath it was found that the time course of anesthesia could not be described by the mathematics of free diffusion. If the cause for this were the complicating effect of the sheath on the course of diffusion, it should be possible to obtain agreement with the theory in nerves without sheath. This has not been possible. It has, it is true, been possible as in experiments with nerves with sheath, to obtain agreement between experimental and theoretical time course, but the minimum concentrations calculated thereby varied in the same way as in the case of experiments with nerves with sheath. The presence of the sheath can therefore not account for the insufficiency of simple diffusion theory to describe the time course of anesthesia.

The dependence of diffusion on the presence of nerve fibers and their distribution.

When the distal part of the nerve is stimulated the anesthetized part of the nerve which determines the time course of anesthesia is the thinnest 10 mm (cf. p. 54) segment closest to the pre-anesthetic electrode (L_{I_s}).

Within the distal part of the sciatic nerve including the distal 15 mm of the anesthetized portion of the nerve there is only one small branch containing 8–10 fibers. Ramus profundus anterior and posterior of the sciatic nerve leave about sixteen mm proximal to the point at which the distal portion of the nerve contacts the local anesthetic. These gross anatomical findings were confirmed by serial sections of the nerve with 0.5 mm between sections*). Therefore the number of fibers must be assumed to be practically identical at the point of stimulation and at the distal part of the nerve in contact with the local anesthetic which determines the time course of diffusion. That this in fact was the case was shown by nerve fiber counts in cross sections of the nerve (Table 14).

Proximal to the point at which the ramus profundus anterior and posterior leave the sciatic nerve, only half of the nerve fibers are activated when the distal end of the nerve is stimulated. Although this portion of the anesthetized part of the nerve is hardly relevant for the time course of diffusion we*) have investigated whether the activated fibers are evenly distributed

Table 14.

Number of large myelinated fibers (10–15 μ in diameter) at the site of stimulation (peroneal and tibial nerves) and in the sciatic nerve immediately distal to the branching point of ramus profundus anterior and posterior. (Right (I) and left (II) nerves from the same frog).

nerve	number of fibers	
	I	II
peroneal	753	734
tibial	556 } 1309	559 } 1293
sciatic	1295	1297

*) experiments performed together with Professor K. G. Wingstrand.

over the cross section or lie in separated bundles according to their origin. The distribution of activated fibers was studied in the following way:

A retrograde degeneration of the distally stimulated nerve fibers was produced by amputating the leg in four frogs below the knee. The tibial and peroneal nerves were cut at a level which corresponded to the site of stimulation in the anesthesia experiments. The frogs were kept for three weeks and the nerves were then fixed and stained according to the method of Marchi (ROMEIS 1948). Degenerated nerve fibers appeared as black spots in the histological sections and could easily be distinguished from other myelinated fibers. The degeneration could be followed proximally in the sciatic nerves. Sections of the nerves proximal to the site at which the stimulating electrodes were placed showed a random distribution of degenerated nerve fibers in the sciatic (Fig. 28). That only a part of the cut fibers appeared degenerated after a period of three weeks is due to the different rates of which degeneration occurs in different fibers and to the fact that only fibers in a certain state of degeneration are stained by the Marchi method. It is, however, unlikely that the state of degeneration is correlated with the distribution of the fibers in the nerve, and it seems justified to conclude *that all stimulated fibers are evenly distributed within the nerve* at the site of the postanesthetic lead.

In the literature no information about this distribution was available on frog nerves. In mammalian nerve it is still unsettled whether the fiber originating from distal branches (peroneal and tibial nerves) are evenly distributed in the sciatic nerve or lie in separate bundles. Some authors have reported an even distribution (FOERSTER, 1929, SUNDERLAND and RAY 1948) others found that the proximal part of the sciatic nerve was still organized in bundles corresponding to the peroneal and tibial nerves (WOHLFART 1938, GUTMANN 1960).

Inside the nerve sheath diffusion is localized in the main to the interstitial space. This implies that:

1) the effective area through which diffusion takes place is considerably smaller than the total cross section of the nerve, and 2) the path of diffusion into the nerve is longer than it would be in an homogeneous medium. If the inhomogeneity did not cause a qualitative alteration in the course of diffusion, this would result solely in a smaller diffusion coefficient than that for diffusion in an homogeneous medium. The figures estimated below indicate the extent to which the presence of fibers in the nerve would diminish the diffusion coefficient:

To facilitate the calculations the nerve fibers were considered as being quadratic with side D, whereas the width of the space between the fibers

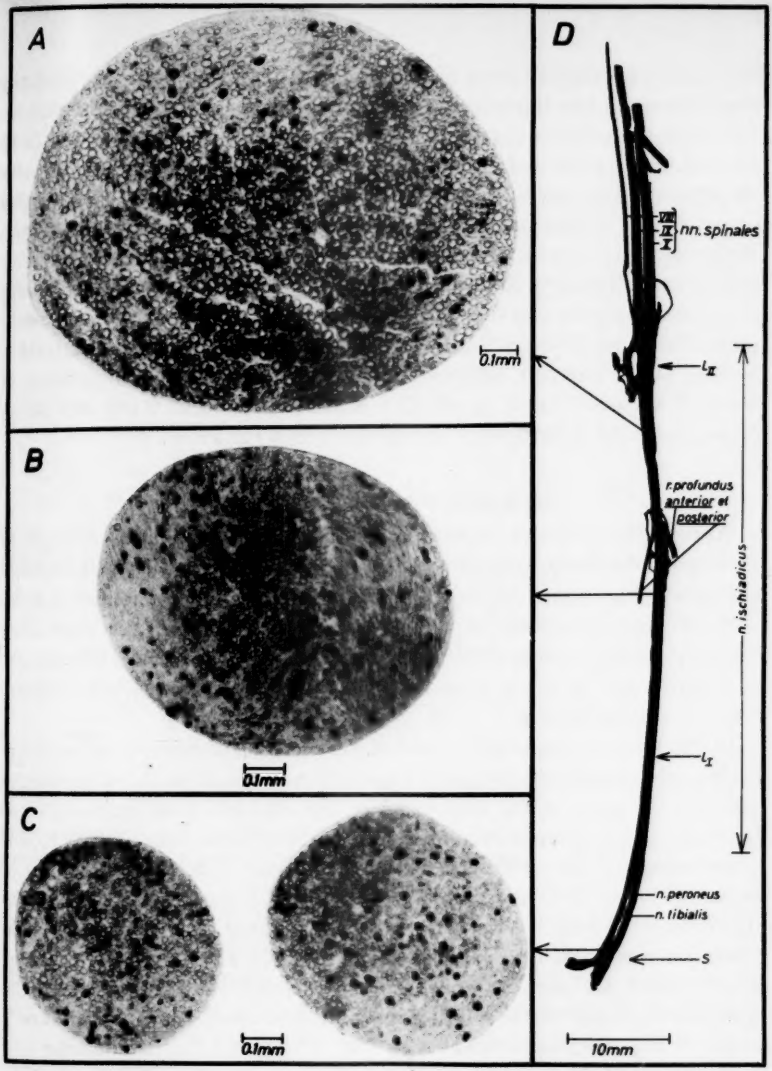


Fig. 28. To show the random distribution of nerve fibers from the tibial and peroneal nerves in the sciatic of the frog by retrograde degeneration.

A-C. Distribution of degenerated nerve fibers (black spots) three weeks after cutting the nerve at *S.* (cf. photograph of osmium stained nerve in *D*).

C. Section about one mm proximal from the site of the stimulating electrodes (*S*),

B. Section distal to the branching of ramus profundus anterior and posterior of the sciatic nerve,

A. Section proximal to the branching of ramus profundus anterior and posterior (fixed and stained according to Marchi's method, 30 μ sections).

L_I site of pre-anesthetic recording electrodes.

L_{II} site of post-anesthetic recording electrodes.

was d. The interstitial space in frog nerve constitutes about one third and the fibers about two thirds of the total volume of the nerve (SHANES 1953 b). It follows that: $D^2/(D+d)^2 = \frac{2}{3}$; $d/(D+d) = 0.18$, in other words only 18 per cent of the nerve's surface can be penetrated by the anesthetic. Thereby the effective diffusion coefficient was diminished to 18 per cent of the value it would have if there were free diffusion through the whole nerve's surface. With this simple model the path of diffusion was $\sqrt{2}$ times longer than with unimpeded diffusion*). Since the time of diffusion increased with the square of the diffusion path and since the effective diffusion coefficient was inversely proportional to diffusion time, the longer diffusion path caused a further halving of the effective diffusion coefficient. In summary, the presence of nerve fibers would cause an effective diffusion coefficient 9 per cent or $\frac{1}{11}$ of the coefficient if there were free diffusion into the nerve.

Hydrolysis of procaine in the nerve.

Whereas the xylocaine molecule is stable and is not split with changes in hydrogen ion concentration or by enzymes, the procaine molecule is unstable and can be hydrolyzed to diethylaminoethanol and paraaminobenzoic acid. This splitting of procaine is hastened in alkaline solution (see page 135). With the velocity constant for hydrolysis given by Terp (1949) the amount of procaine split in one hour at 20°C would be 0.1 per cent at pH 7.35 and 0.25 per cent at pH 7.8.

In the organism, especially in serum, the splitting of procaine occurs more rapidly on account of the presence of procaine esterase. It has been questioned whether the nerve tissue also contains this esterase. NORDQUIST (1952 a) believed to have demonstrated it, in disagreement with SKOU (1954). NORDQUIST measured the splitting of 0.08 mg procaine added to 67–178 mg of nerve tissue. At 20°C and pH 7.3 he found that 1 g of nerve tissue hydrolyzed about 20×10^{-6} g procaine, or 1.5–4 per cent of the procaine in an hour. The non-enzymatic splitting calculated from TERP's velocity constant would be 0.1 per cent per hour. SKOU (1954) did not find an increase in splitting when a procaine solution was added to nerve tissue. From SKOU's data (70 mg procaine, 2 hours, pH 7.35, 37°C), the reaction scheme and TERP's velocity constant, a splitting of 1.4 per cent per hour would be expected; SKOU (1954) found experimentally a splitting of 1.2 per cent per hour. The question as to whether procaine is split enzymatically in nerve must therefore be considered unsettled. From a mathematical analysis of

*) The increase in the path of diffusion was estimated as the ratio between the length of two sides of a quadratic fiber ($2D$) and the length of a diagonal ($D \times \sqrt{2}$).

the influence of the splitting of procaine on the course of diffusion of procaine into nerve ROSENFALCK concluded, that the spontaneous hydrolysis of procaine is so small that it would not appreciably affect the time course of procaine's penetration into the nerve (see Appendix 2). The same would be the case even if the splitting should be 10 times greater than indicated above on account of the presence of procaine esterase in nerve.

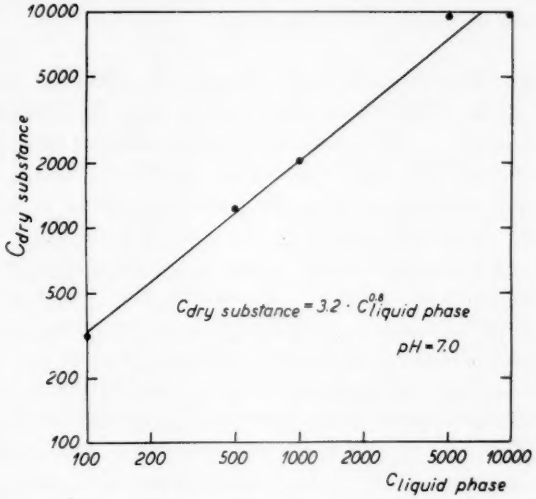


Fig. 29. Distribution of procaine between the dry and liquid phases of nerve (SKOU 1954: Table 10) pH 7.0, 37° C.
Ordinate: concentration of procaine in the dry phase (mg %).
Abscissa: concentration of procaine in the liquid phase (mg %).

The binding of the anesthetic to nerve tissue.

The anesthetic penetrates into the nerve via the interfibrillar space in a watery solution. Since the active base component of the anesthetic is soluble in lipids, a portion of it will be dissolved in the lipids of the nerve fibers. If the binding is firm and occurs instantaneously the expression for simple diffusion is applicable with a diffusion coefficient less than if there were no binding (CRANK, 1956).

Investigations as to the binding of xylocaine to nerve tissue have not been carried out, but SKOU (1954) investigated the distribution of procaine between the dry and fluid components of peripheral nerve. The experiments were carried out on nerves from the cauda equina of cows at pH 7.0 and

37° C (Figure 29, from SKOU's Table 10, 1954). Procaine was bound to the dry phase according to the following relationship:

$$C_{\text{dry phase}} = 3.2 \times C^{0.8}_{\text{watery phase}}$$

in other words procaine binding to the dry phase of nerve is proportional to the concentration in the watery phase to the power 0.8. According to CRANK (1956) this distribution would bring about an effective diffusion coefficient $\frac{1}{4}$ of that which obtains with free diffusion.

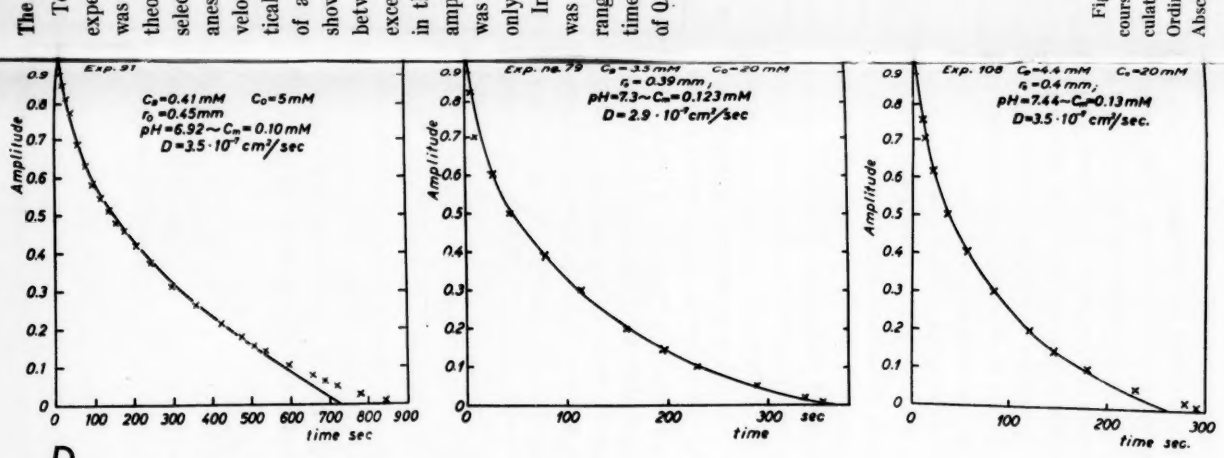
Conclusion: The above discussion concerns the effect of various factors which could be imagined to invalidate the use of the mathematics of simple diffusion to calculate a minimum concentration identical with that determined experimentally. The result was that for most of the factors there should be no principal objection to using the diffusion formula. It was, however, assumed that the inhomogeneity of the nerve did not affect the relative time course of anesthesia and that the binding of the anesthetic to the nerve tissue was firm and instantaneous. In addition the theoretical description does not consider the effect of subminimal concentrations on the action potential amplitude. The lack of agreement between theory and experiment may therefore be ascribed to these factors. To what extent they can account for the difference between the calculated and the experimentally determined minimum concentration remains an open question.

Chapter 6

DETERMINATION OF A VELOCITY FACTOR FOR THE TIME COURSE OF ANESTHESIA FROM DIFFUSION THEORY AND THE EXPERIMENTALLY DETERMINED MINIMUM CONCENTRATIONS

It was not possible, from the time course of anesthesia and the mathematics of free diffusion to determine both a minimum concentration of the anesthetic identical with that measured directly and a diffusion coefficient, identical in experiments with different concentrations (Chapter 5). The question remains, whether an empirical expression can be found which allows prediction of the time course of anesthesia at different concentrations and on nerves with different diameters. The effect of a given local anesthetic on the single nerve fiber is adequately described by the minimum concentration required to block the fiber. In whole nerve there is an additional factor in that it takes time for anesthesia to develop. The time course of anesthesia is not a simple function of either the outer concentration or the minimum concentration. In the following an attempt is made to characterize the rapidity with which the anesthetic penetrates into the nerve by introducing a "velocity factor". This would allow prediction of the time course of anesthesia with different concentrations and for nerves of different diameter. It would facilitate the comparison of the effect of a given anesthetic under different conditions. If, for example changes are introduced in the local anesthetic or in the tissue which might change its penetration into the nerve, this would appear in different velocity factors. Furthermore – aside from the minimum concentration – a velocity factor might be used in evaluating the effectiveness of different anesthetics on whole nerves.

Since the anesthetic penetrates into the nerve by diffusion, it seems near at hand to test the possibility of predicting the time course of anesthesia by means of the differential equation for free diffusion in a cylinder and the experimentally determined minimum concentration. D in equation (2) page 85 would in this case be considered a common *velocity factor* for the time course of anesthesia at various concentrations of the local anesthetic.



D

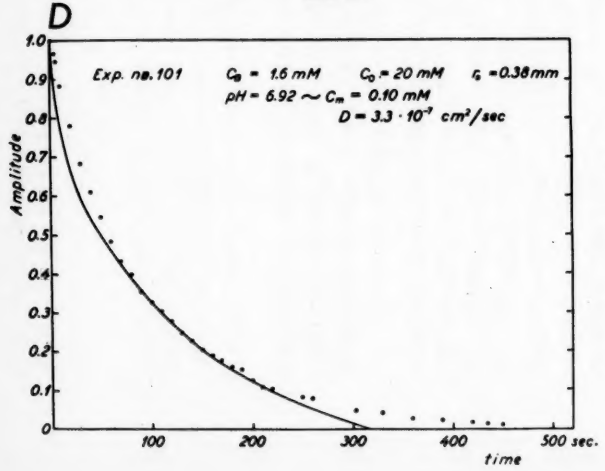


Fig. 30. Examples of the agreement (*A*, *B*, *C*) between experimental and calculated (solid lines) time course of xylocaine anesthesia (nerves with sheath).

Ordinate: action potential amplitude in units of the amplitude in Ringer's.

Abscissa: time in seconds after application of the anesthetic. (Note different time scales).

The theoretical time course was calculated from diffusion theory and the experimental minimum concentrations.

D is an example of experiments in which the calculated decrease in action potential amplitude initially was slightly larger than the experimental.

The determination of a velocity factor for xylocaine in nerves with sheath.

To test the possibility of introducing a common velocity factor D for experiments with different concentrations of the anesthetic the factor D was determined for each experiment in the following way: Among the theoretically calculated time courses of anesthesia (Fig. 26 A) the one is selected which has the same ratio between the base concentration of the anesthetic and the minimum concentration as the experimental curve. The velocity factor is then determined as that value of D for which the theoretical curve is in closest agreement with the experimental curve. Examples of agreement between the experimental and the theoretical curves are shown in Figure 30 A–C. In 30 of 46 experiments with xylocaine the difference between the experimental and the theoretical amplitude values did not exceed 6 per cent in the amplitude range 1–0.2 (initial amplitude = 1), and in the amplitude range 0.2–0 the maximum amplitude deviation was 0.06 amplitude units (Table 15). When the deviation was expressed in time there was a significant difference between the experimental and theoretical courses only in the beginning of the course of anesthesia.

In eight experiments (example in Figure 30 D) the maximal difference was 9 per cent between the experimental and theoretical amplitude in the range 1–0.2. When the deviation for these experiments was expressed in time the deviation in the beginning of the course of anesthesia at an amplitude of 0.75, was twice as great as the deviation in the 30 experiments described

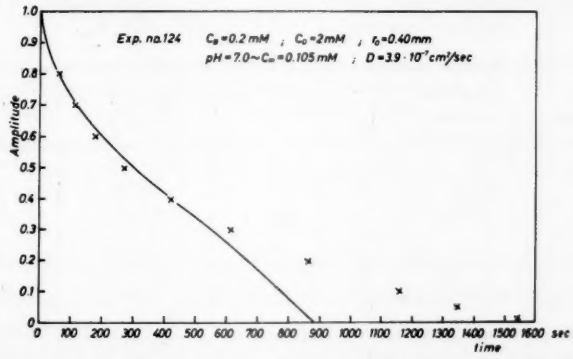


Fig. 31. Example of deviation of the experimental from the calculated (solid line) time course of xylocaine anesthesia (nerve with sheath). The theoretical time course was calculated from diffusion theory and the experimental minimum concentration.
Ordinate: action potential amplitude in units of the amplitude in Ringer's.
Abscissa: time in seconds after application of the anesthetic.

Table 15.

Differences between the experimental and calculated time courses of anesthesia in 46 experiments with xylocaine. The theoretical course calculated from diffusion theory and the experimental minimum concentration.

Difference in per cent between the experimental and calculated time to various levels of action potential amplitude.

number of experiments	amplitude level	0.75	0.35	0.10	0
30	difference	30	0	0	15
	S.D.	27	5	7	19
8	difference	65	— 4	— 1	16
	S.D.	24	14	10	18
3	difference	110–140	0–12	— 6–0	0–25
5	difference	0–8	0–20	15–135	50–260

Difference between experimental and calculated action potential amplitude in various amplitude ranges (in units of the initial amplitude).

number of experiments	amplitude range	1–0.5	0.5–0.2	0.2–0
30	difference	0.045	0.023	0.030
	S.D.	0.020	0.015	0.010
8	difference	0.085	0.030	0.034
	S.D.	0.020	0.015	0.020
3	difference	0.01–0.14	0.01–0.04	0.02–0.03
5	difference	0.01–0.03	0.02–0.13	0.06–0.20

above, whereas at the lower amplitude levels there was the same agreement as in the 30 experiments described above.

In the remaining eight experiments there was poor agreement between the experimental and the theoretical course: In five experiments, with base concentrations less than two times the minimum concentration, the last portion of the experimental course of anesthesia was clearly slower than the theoretical (Figure 31). In three experiments there was a marked deviation in the beginning.

The mean value of the velocity factor for xylocaine in the 30 experiments with agreement between the experimental and theoretical course of anesthesia was $2.9 \times 10^{-7} \pm 0.2 \times 10^{-7}$ cm²/sec and for all 46 experiments $3.0 \times 10^{-7} \pm 0.15 \times 10^{-7}$ cm²/sec ($S.D. = 35$ per cent).

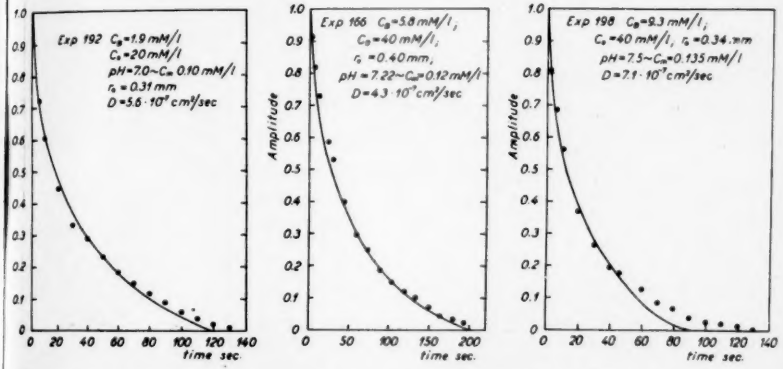


Fig. 32. Examples of the agreement between experimental and calculated (solid lines) time course of xylocaine anesthesia (nerves without sheath).
Ordinate: action potential amplitude in units of the amplitude in Ringer's.
Abscissa: time in seconds after application of the anesthetic. (Note different time scales).
The theoretical time course was calculated from diffusion theory and the experimental minimum concentration.

Determination of the velocity factor for xylocaine in nerves without sheath.

In experiments on nerves without sheath agreement was found between the experimental and the calculated course of anesthesia for action potential amplitudes above 10–15 per cent of the initial value when the experimentally found minimum concentrations was used; when the amplitude was below 10–15 per cent the experimental time course was in general slightly slower than the theoretical (Figure 32).

In 14 of the 16 experiments carried out at a base concentration greater

than the minimum concentration the deviation in the amplitude range 1–0.5 did not exceed 0.06 amplitude units or 8 per cent. In the amplitude range 0.5–0.2 the maximal deviation was 0.03 amplitude units or 7 per cent. In the amplitude range 0.2–0 the maximum deviation did not exceed 0.024 amplitude units. In two experiments at xylocaine base concentrations 2-3 times the minimum concentration (nos. 125 and 189) there was poor agreement between the theoretical and experimental curves.

The mean value of the velocity factor for xylocaine in the 14 experiments on nerves without sheath with agreement between theory and experiment was $8.9 \times 10^{-7} \pm 0.8 \times 10^{-7}$ cm²/sec (*S.D.* = 34 per cent).

Determination of the velocity factor for procaine in nerves with sheath.

In 13 of 16 experiments with procaine there was agreement between the experimental and theoretical time course in the range 0.35–0 amplitude units with a maximum deviation of 0.03 amplitude units (Fig. 33 A, B, C). In the initial course of the anesthesia, i.e. the amplitude range 1–0.35 the experimental course in these 13 experiments was slower than the theoretical. The maximum amplitude deviation in this range averaged 0.13 amplitude units corresponding to 25 per cent. Expressed as deviation in time, the experimental time interval required for a reduction in action potential amplitude to 0.75 amplitude units was on the average twice as long as the theoretical (S.D. 34 per cent); in the amplitude range 0.35 to total block there were no significant differences between theoretical and experimental curves.

In three experiments with base concentrations lower than 3.5 times the minimum concentration (Fig. 33 D) the last portion of the experimental course progressed considerably slower than the theoretical. Thus, the agreement between theory and experimental findings was not as close in the case of procaine as in the case of xylocaine.

The velocity factor determined for the 13 experiments with procaine where agreement with theory was obtained in the later part of the anesthesia averaged $2.1 \times 10^{-7} \pm 0.1 \times 10^{-7}$ cm²/sec. (S.D. = 19 per cent).

Discussion. In the previous chapter it was shown that the model representing free diffusion of the anesthetic substance was at variance with the experimental findings, in that the minimum concentrations calculated from the model differed from those found experimentally. Nor were the calculated diffusion coefficients the same at all concentrations of the anesthetic. On the other hand from the differential equation describing free diffusion in a

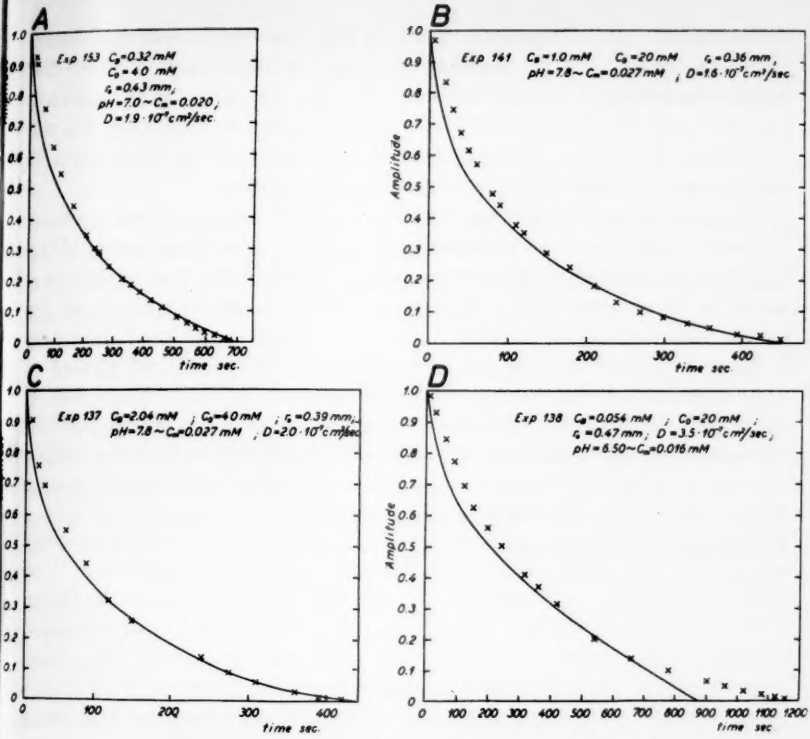


Fig. 33. Comparison of the experimental and calculated (solid lines) time course of procaine anesthesia in nerves with sheath.
Ordinate: action potential amplitude in units of the amplitude in Ringer's.
Abscissa: time in seconds after application of the anesthetic. (Note different time scales).
The theoretical time course was calculated from diffusion theory and the experimental minimum concentrations. *A*, *B*, *C* are examples of agreement between experiment and theory in the last portion of the course of anesthesia. *D* is an example of a disagreement between theory and experiment.

cylinder, the experimentally determined minimum concentrations at the pH of the solution and the experimental time courses of anesthesia, a common velocity factor D for anesthesia could be calculated which allowed prediction of a major portion of the time course of anesthesia at different concentrations.

With **xylocaine** the time course could be predicted until the action potential amplitude was redueed to 15–20 per cent of its initial value, on nerves with and without sheath and with concentrations 3 to 90 times the minimum

concentration. The experimental blocking time was longer than that determined theoretically. With **procaine** the range of amplitudes in which the velocity factor could be used was narrower than for xylocaine in that only the later part of the time course of anesthesia could be predicted, i.e. the range from 35 per cent of the amplitude in Ringer's to full block, for concentrations of 3.5–80 times the minimum concentration.

It appears from these findings that the empirical expression derived from diffusion theory combined with the experimentally determined values of the minimum concentration allowed prediction of the major part of the time course of local anesthesia with xylocaine in different concentrations for nerves with and without sheaths. The faster action of the local anesthetic in nerves without sheaths appeared as an increase in the velocity factor.

As illustrated by the findings with xylocaine and procaine, prediction of the time course of local anesthesia with different anesthetics meets the difficulty that the agreement between experimental and calculated course may occur at different degrees of anesthesia. Therefore, under these circumstances the velocity factor does not characterize differences in the action of different local anesthetics.

Chapter 7

THE RESTITUTION OF THE NERVE ACTION POTENTIAL AFTER ANESTHESIA

This chapter deals with the time course of restitution of the nerve action potential after local anesthesia with various concentrations of xylocaine and procaine base. Furthermore, an attempt will be made to determine the extent to which the course of restitution can be described in terms of diffusion of the anesthetic out of the nerve.

When the nerve is just fully blocked the anesthetic is distributed in the nerve as illustrated in Fig. 34 a. The most central fiber has just been exposed to a concentration equal to the minimum concentration. Maintaining the anesthetic around the nerve, diffusion will continue into the nerve until the concentration in the nerve is the same as that outside it (Fig. 34 b). Replacing the bathing solution with Ringer's solution, the anesthetic diffuses out of the nerve. When the concentration in the peripheral portions of the nerve falls just below the minimum concentration (Fig. 34 c) the first signs of activity appears. Investigations along these lines should allow a mathematical analysis of the time course of diffusion. There is however the disadvantage that there is no criterion to indicate when the nerve is fully saturated with the anesthetic though the time required for full saturation could be estimated theoretically.

Instead of waiting for full saturation anesthesia was arrested when the most central fibers were blocked (Fig. 34 a). This time can be measured exactly since it coincides with the time at which the action potential disappears. This has the consequence however that further diffusion is complicated in that the anesthetic diffuses both into and out of the nerve. At a given time after substitution of the anesthetic with Ringer's and while there is still full anesthesia the conditions are approximately as shown in Fig. 34 d (see also Fig. 45). At the first sign of activity the distribution of the anesthetic within the nerve should be as shown in Fig. 34 e.

An example of the restitution of the nerve action potential amplitude, the xylocaine solution having been replaced by Ringer's solution when the

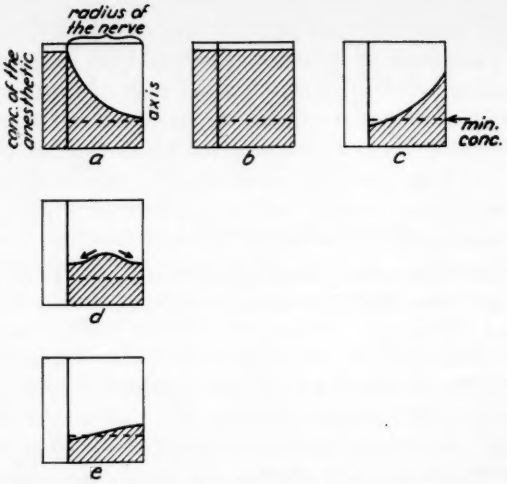


Fig. 34. Schematic illustration of the distribution of the anesthetic within the nerve at various times after its application and removal.

a. the distribution at the time when the nerve is just blocked by the anesthetic.
b. the nerve is fully saturated with the outer concentration of anesthetic.
c. the saturated nerve is placed in Ringer's and diffusion takes place out of the nerve. When the concentration at the nerve surface is below the minimum concentration, the nerve begins to respond again.
d. the anesthetic solution is substituted with Ringer's when the nerve is just fully anesthetized (cf. a). Initially the anesthetic diffuses both into and out of the nerve.
e. later, when the peripheral concentration is below the minimum concentration the first evidence of nerve activity is obtained.

nerve was just fully blocked, is illustrated in Fig. 35 (see also Fig. 46). About 25 minutes elapsed before the nerve developed an action potential. After the action potential reappered it increased in amplitude to about 5 per cent of the normal value within 10–15 minutes. During the next 20 minutes it increased nearly rectilinearly to about 80 per cent of normal amplitude, to approach 100 per cent within the next 30 minutes.

The onset of restitution was defined as the time interval after replacement of the anesthetic with Ringer's until the amplitude was restored to 1 per cent of the normal amplitude ($T_{0.01}$). The time interval until the amplitude attained 100 per cent was difficult to determine because of the diminished slope late in the course of restitution. Therefore I chose the time interval at which the amplitude reached 50 per cent of normal amplitude ($T_{0.5}$) as a measure of the duration of restitution. To compensate for differences in nerve thickness these time intervals were divided by the square of the radius.

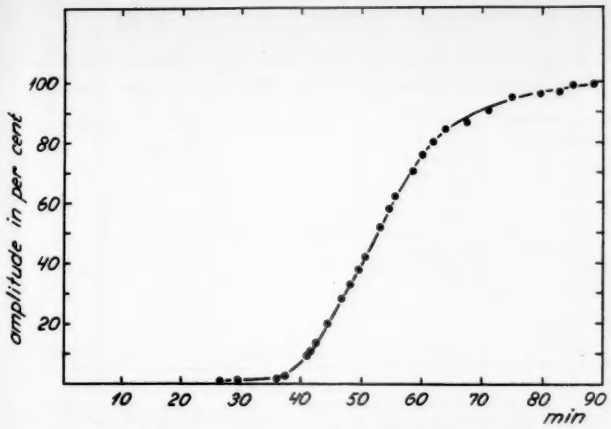


Fig. 35. Restitution of the nerve action potential amplitude after anesthesia with xylocaine (C_B: 3.7 mM, nerve radius: 0.37 mm, 22° C, nerve without sheath). Ordinate: amplitude in per cent of the amplitude in Ringer's before anesthesia. Abscissa: time in minutes after substitution of xylocaine with Ringer's.

Restitution of the action potential after anesthesia of nerves with sheath.

In 17 experiments the restitution of the nerve action potential was studied after anesthesia with various concentrations of xylocaine and procaine.

After the blocked nerve was replaced in Ringer's solution it took 10–90 minutes for the action potential to return to 1 per cent of its normal value except when the concentration of anesthetic was very close to the minimum concentration. The time required for the action potential to regain 50 per cent of its normal amplitude was about twice as long as the time to 50 per cent anesthesia when the concentrations were low (experiments 139 and 156); with the largest concentrations used (experiments 137 and 162) restitution took 150–230 times as long as anesthesia. These differences can be explained in part by the course of diffusion (cf. p. 150).

The correlation between restitution times (to onset and to 50 per cent restitution) and concentration of xylocaine and procaine base was obtained in experiments with different concentrations of the anesthetics (Tables 16, 17, Fig. 36 a, b). In the xylocaine experiments the base concentrations of the anesthetic was varied between 0.15 mM and 13.7 mM. In procaine a group of 5 experiments were performed with concentrations of 1 to 1.1 mM and allowed a calculation of the standard deviation of the time to the onset of restitution ($T_{0.01}/r_0^2$). This was 10 per cent.

Table 16.

Restitution of action potential amplitude after anesthesia with different xylocaine concentrations and pH (nerves with sheath).

experiment no.	hydrochloride concentration (C_0) mM	pH	base concentration (C_B) mM	temperature °C	nerve radius (r_0) mm	$T_{0.01}$ minutes	$\frac{T_{0.01}}{r_0^2}$ sec/cm²	$T_{0.5}$ minutes	$\frac{T_{0.5}}{r_0^2}$ sec/cm²
156	2	6.98	0.15	22.7	0.39	0.75	2.90×10^{4}	29.0	1.12×10^{6}
136	5	6.84	0.33	23.5	0.45	10.5	3.05×10^{5}	29.3	8.56×10^{5}
157	20	6.70	1.00	23.4	0.40	16.0	5.85×10^{5}	54.0	1.97×10^{6}
154	20	7.02	2.00	22.7	0.40	19.75	7.32×10^{5}	54.0	2.0×10^{6}
159	40	6.90	3.00	22.8	0.44	22.0	6.74×10^{5}	77.5	2.37×10^{6}
161	20	7.57	5.5	23.0	0.46	40.75	1.15×10^{6}	99.3	2.8×10^{6}
160	40	7.40	8.1	22.2	0.44	52.0	1.61×10^{6}	131.0	4.07×10^{6}
162	40	7.72	13.7	22.1	0.43	88.0	2.86×10^{6}	194.0	6.3×10^{6}

Table 17.

Restitution of action potential amplitude after anesthesia with different procaine concentrations and pH (nerves with sheath).

139	20	6.00	0.017	22.2	0.38	1.8	7.80×10^{4}	34.0	1.44×10^{6}
138	20	6.50	0.054	23.3	0.47	6.2	1.72×10^{5}	31.0	8.58×10^{5}
135	40	7.00	0.33	24.5	0.45	17.7	5.20×10^{5}	42.5	1.26×10^{6}
140	20	7.80	1.0	25.6	0.35	33.0	1.64×10^{6}	65.0	3.22×10^{6}
146	20	7.80	1.0	25.3	0.43	43.5	1.44×10^{6}	90.0	2.9×10^{6}
150	20	7.80	1.0	23.9	0.45	63.0	1.83×10^{6}	113.5	3.3×10^{6}
149	20	7.80	1.0	24.5	0.41	50.5	1.80×10^{6}	87.0	3.1×10^{6}
147	40	7.55	1.1	25.5	0.40	50.0	1.84×10^{6}	88.0	3.24×10^{6}
137	40	7.80	2.0	23.2	0.39	75.0	2.90×10^{6}	176.0	6.82×10^{6}

$T_{0.01}$ and $T_{0.5}$ are the times from replacement of the anesthetic by Ringer's till the action potential amplitude was 1 per cent and 50 per cent of the amplitude in Ringer's before anesthesia.

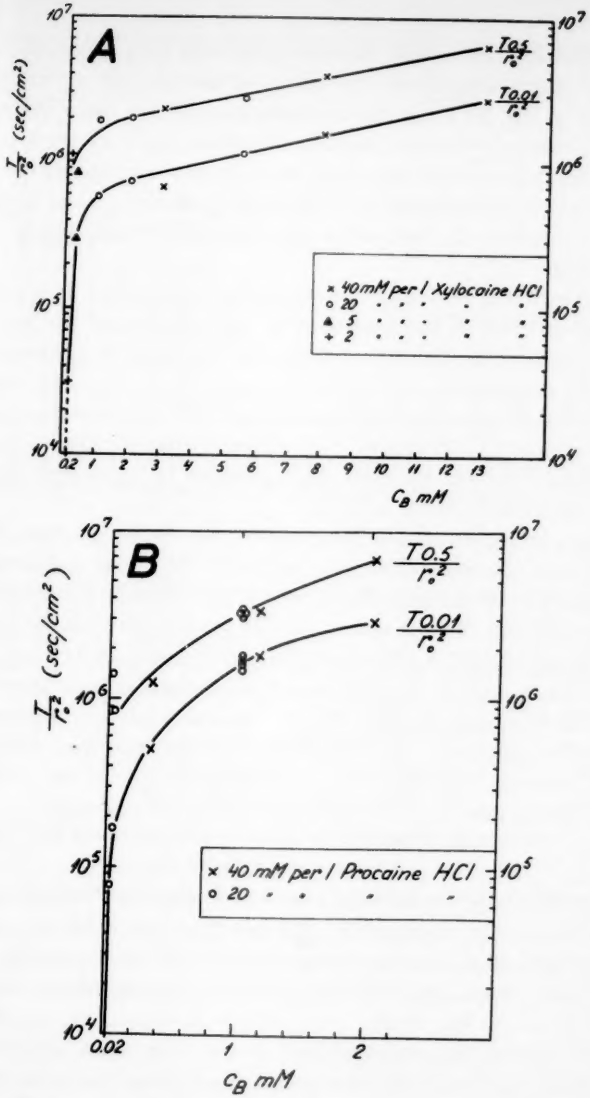


Fig. 36. Restitution time for the nerve action potential amplitude after anesthesia with different xylocaine (*A*) and procaine (*B*) base concentrations (C_B). Nerves with sheath. (Temperature range: 22.1–25.6° C).

Ordinate: time after substitution of the anesthetic by Ringer's (at the moment when the nerve is just blocked) until the amplitude regains 1 per cent ($T_{0.01}$) and 50 per cent ($T_{0.5}$) of normal. The time is compensated for variations in nerve thickness by division by the square of the radius (r_0) (sec/cm²) (log scale).

Abscissa: base concentration of the anesthetic (mM).

The symbols indicate hydrochloride concentrations.

The restitution time varied systematically with base concentration independent of the hydrochloride concentration and the pH at which it was obtained (Fig. 36). As a function of acid concentration there was no systematic variation in restitution time. In Figure 36 consideration is not taken of the various pH values at which the experiments were carried out. Correcting for these and expressing the base concentrations in units of the minimum concentration, the restitution time increased systematically with the concentration.

Another way of determining the minimum concentration is to reduce the outer concentration of the anesthetic to the extent that the first sign of activity appeared at the earliest time after substitution of the anesthetic by Ringer's solution.

In Fig. 36 there are only a few experiments with base concentrations close to the minimum concentration. The minimum concentrations obtained by extrapolation were for xylocaine and procaine in agreement with those found experimentally (cf. p. 60).

That the restitution time in fact is very short (a few minutes) when the concentration of the anesthetic was close to the minimum concentration was also apparent in the experiments in which the minimum concentration was determined directly. The nerves which were blocked with "minimum concentration" (4 nerves with a xylocaine base concentration of 0.04 mM, pH 6.0; 6 nerves with a procaine base concentration of 0.013 mM at pH 6.12 and 4 nerves with 0.021 mM at pH 7.08) gave a measurable action potential 1–2 minutes after the nerve was transferred from the anesthetic solution into Ringer's solution. That the action potential did not appear immediately might be due to binding of the anesthetic to the nerve tissue.

Restitution of the action potential after anesthesia of nerves without sheath.

Fig. 37 shows the values of $T_{0.01}/r_0^2$ and $T_{0.5}/r_0^2$ from 10 experiments on nerves without sheath as a function of the concentration of xylocaine base used for anesthesia. The solid lines refer to experiments on nerves with intact sheaths (see Fig. 36 a).

The restitution time was the same in nerves without as in those with sheath. The same result was obtained by TRUANT and LANZONI (1952). The time course of restitution was not determined by diffusion alone, but influenced by binding of the anesthetic to the nerve tissue (cf. Appendix 3). That the removal of the nerve sheath did not affect the restitution times, indicates that the effect of binding is greater than the delaying effect of the sheath on the diffusion of the anesthetic out of the nerve.

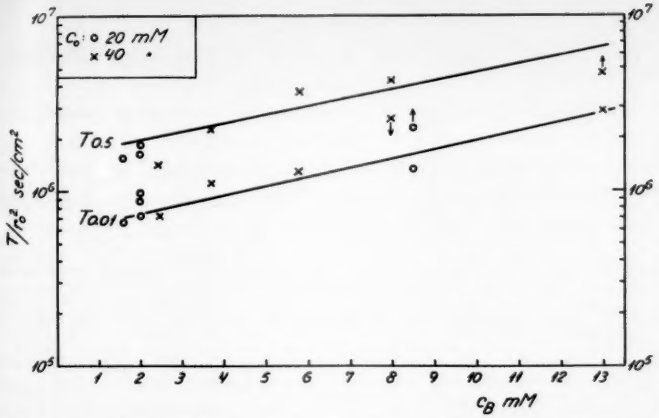


Fig. 37. The restitution of action potential amplitude after anesthesia with various xylocaine base concentrations (C_B) in nerves without sheath (temperature range: 20.1–25.3° C).

Ordinate: time after substitution of xylocaine by Ringer's until amplitude regains 1 per cent ($T_{0.01}$) and 50 per cent ($T_{0.5}$) of normal. The time is compensated for variations in nerve thickness by division by the square of the radius (r_0) (sec/cm²) (log scale).

Abscissa: xylocaine base concentration (mM).

The solid lines indicate the average restitution times for nerves with sheath (cf. Fig. 36). The symbols refer to different hydrochloride concentrations.

Comparison between restitution times after anesthesia with xylocaine and procaine.

In Table 18 the restitution times to 1 and 50 per cent amplitude are compared for 2 and 1 mM xylocaine and procaine *base*. The nerve impulse was restored about three times faster after xylocaine than after procaine anesthesia. The difference was somewhat more pronounced for the time to 1 than to 50 per cent amplitude restitution.

The restitution times may also be compared after anesthesia with the same concentrations of xylocaine and procaine *hydrochloride at the same pH*. Considering for example 40 mM xylocaine and procaine hydrochloride at pH 7.3 this corresponds to base concentrations of 6.6 and 0.65 mM respectively (Table 19). There was only a slight difference in the restitution times in that the procaine-anesthetized nerve was restored about one and a half times faster than the xylocaine-anesthetized.

Table 18.

Restitution after anesthesia with xylocaine and procaine with the same base concentration.

base concentration (C_B) mM		$\frac{T_{0.01}}{r_0^2}$ sec/cm²	$\frac{T_{0.01}\text{ procaine}}{T_{0.01}\text{ xylocaine}}$	$\frac{T_{0.5}}{r_0^2}$ sec/cm²	$\frac{T_{0.5}\text{ procaine}}{T_{0.5}\text{ xylocaine}}$
2	procaine	2.9×10^6	3.8	6.8×10^6	3.4
	xylocaine	7.6×10^5		2.0×10^6	
1	procaine	1.6×10^6	2.8	3.2×10^6	2.0
	xylocaine	5.8×10^5		1.6×10^6 *)	

*) interpolated value from the curve Fig. 36A.

Table 19.

Restitution after anesthesia with xylocaine and procaine with the same hydrochloride concentration.

	base concentration at pH 7.3 (C_B) mM	$\frac{T_{0.01}}{r_0^2}$ sec/cm²	$\frac{T_{0.01}\text{ procaine}}{T_{0.01}\text{ xylocaine}}$	$\frac{T_{0.5}}{r_0^2}$ sec/cm²	$\frac{T_{0.5}\text{ procaine}}{T_{0.5}\text{ xylocaine}}$
procaine HCl 40 mM	0.65	1.0×10^6	0.77	2.1×10^6	0.64
xylocaine HCl 40 mM	6.6	1.3×10^6		3.3×10^6	

The restitution times have been interpolated from the curves of Fig. 36.

Discussion. Clinical studies have indicated that there is a correlation between the concentration of the anesthetic and the restitution time after anesthesia. With the wheal test and corneal reflex in rabbits as indicators there was a logarithmic relation between the duration of anesthesia and the

concentration of the anesthetic (SINHA 1939 and MIESCHER 1941). GOLDBERG (1944) and BJÖRN (1946) found that the larger the concentration of anesthetic the more rapid the onset of anesthesia and the longer its duration.

I have found the restitution time both for xylocaine and for procaine to increase with the base concentration as has been shown previously by GARDNER, SEMB and GRAHAM (1934). The orderliness of this relationship is a further indication that it is the free base which is the active anesthetic component.

BENNETT et al. (1942) and TRUANT and LANZONI (1952) observed, as I have found, that the restitution time was considerably longer than the time to nerve block. They consider that this cannot be explained by the course of diffusion, but assume that the anesthetic is bound to nerve tissue thus delaying diffusion out of the nerve.

During the development of the anesthesia there is usually a large concentration gradient between the outer solution and the interior of the nerve so that diffusion progresses rapidly. During restitution the gradient rapidly diminishes between the interior of the nerve and the surroundings and diffusion progresses more slowly. If the anesthetic is removed just after block is achieved, diffusion of the anesthetic continues from the periphery to the centre of the nerve and thereby the diffusion time out of the nerve is further increased.

In addition to the concentration gradient the diffusion of the anesthetic out of the nerve depends on its radius, the velocity factor and the minimum concentration. The greater the minimum concentration of the anesthetic the more rapidly is the fall in the concentration peripherally below the minimum concentration and thereby the peripheral fibers regain their ability to conduct impulses. That the experimental time course of restitution was *qualitatively* determined by these factors may be seen by comparing the restitution times after anesthesia with the same amounts of xylocaine and of procaine *base*. Whereas the size of the velocity factor is of the same order, xylocaine base had a higher minimum concentration than procaine base, and the nerve was restituted fastest after xylocaine anesthesia (Table 18).

The question is, however, whether minimum concentration, base concentration, nerve radius and velocity factor which in the main can account for the time course of the reduction in amplitude (Chapter 6), can account quantitatively for the experimentally found time course of restitution.

ROSENFALCK found that restitution took considerably longer than to be expected from simple diffusion theory (Appendix 3, Fig. 47). Thus the time from substitution of the solution of the local anesthetic with Ringer's until the action potential regained 1 per cent of the initial amplitude was 20–40

times longer than to be expected if the time course of restitution were determined solely by diffusion of the anesthetic, using the values for velocity factor and minimum concentration found in chapter 6 and 4. Considering the times until the amplitude returned to 50 per cent of the initial amplitude in Ringer's, the experimentally found times were 2–3 times longer than those theoretically to be expected. The later onset of restitution than theoretically expected might be explained as suggested by BENNETT et al. (1942) and TRUANT and LANZONI (1952), by binding of the anesthetic to nerve tissue, delaying diffusion of the anesthetic out of the nerve.

Considering the restitution times after anesthesia with the same amounts of xylocaine and procaine *hydrochloride* at the same pH, it was found that the nerve was restituted slightly more rapidly after procaine anesthesia. The cause for this must be, that the concentration of procaine base was less than of xylocaine base, the restitution time depending on the base concentration.

Chapter 8

THE EFFECT OF LOCAL ANESTHETICS ON THE CONDUCTION VELOCITY OF THE NERVE ACTION POTENTIAL

Kato (1926), Tamura (1928) and Toman, Woodbury and Woodbury (1947) demonstrated that the conduction velocity decreases in that portion of a nerve subjected to cocaine and procaine as the anesthesia progresses. I have confirmed these findings for procaine and have found a similar relationship for xylocaine (Figs. 38, 39).

There were two possible causes for this decrease in conduction velocity:

1) the conduction velocity was diminished in all fibers in contact with concentrations of the local anesthetic below the minimum concentration.

2) the most rapid fibers were affected first and were therefore blocked before the slow fibers.

To decide between these possibilities the conduction velocity was determined in the postanesthetic portion of the nerve. A third pair of recording electrodes was placed on the nerve proximal to those from which the postanesthetic recording was taken. If the conduction velocity were diminished on account of a selective block of the fast fibers, one would expect to find a reduction in conduction velocity both in the anesthetized and the postanesthetic section of the nerve. If the conduction velocity were diminished in fibers which still conducted but were affected by subminimal concentrations of the anesthetic, these fibers would again conduct with normal velocity in the postanesthetic section of the nerve.

The result of three experiments with 0.25 mM xylocaine base was that the conduction velocity measured in the postanesthetic section of the nerve was the same as the conduction velocity measured in Ringer's, indicating that the local anesthetic in subminimal concentrations reduces the conduction velocity of the single fiber. Similar results were obtained with ether (Boruttau and Frölich 1904) the conduction velocity being diminished in the anesthetized section of nerve and normal distal and proximal to the affected nerve section.

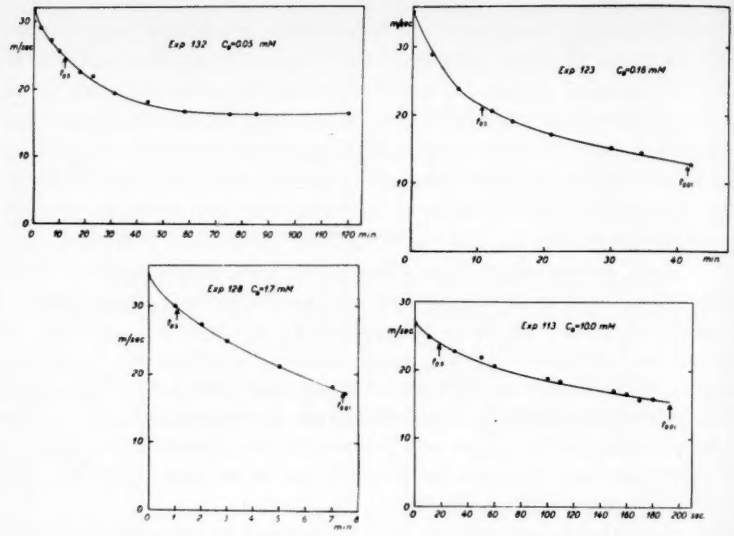


Fig. 38. Examples of the diminution in conduction velocity with time during anesthesia.
Ordinate: conduction velocity in m/sec.
Abscissa: time after application of xylocaine. (Note different time scales).
C_B indicates the xylocain base concentration, $t_{0.5}$ and $t_{0.01}$ indicate the times at which the amplitude was reduced to 50 and 1 per cent of the pre-anesthetic value. (Corrected to temperature 22° C).

Experiments are described below in which the diminution in action potential conduction velocity was determined as related to:

1) the degree of anesthesia of the nerve,
2) the base concentration of the anesthetic and
3) anesthesia with xylocaine and procaine.

The diminution in conduction velocity during the development of anesthesia.

In Table 20 are given the conduction velocities in the anesthetized section of the nerve measured when the action potential amplitude amounted to 50 and 1 per cent of its initial value. The velocity decreased most rapidly immediately after application of the anesthetic and more slowly thereafter

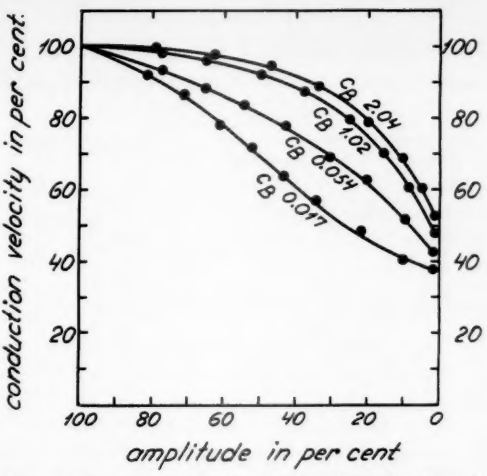


Fig. 39. Diminution in conduction velocity as a function of progressing anesthesia for different concentrations of procaine base. C_B in mM indicated on the curves (22° C).
Ordinate: conduction velocity in per cent of the value in Ringer's.
Abscissa: nerve action potential amplitude in per cent of the value in Ringer's.

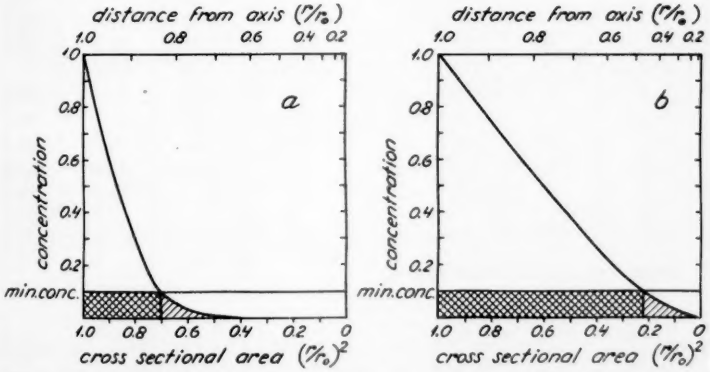


Fig. 40. Diagram to illustrate the effect of subminimal concentrations on nerve conduction velocity at different times after application of the anesthetic to the nerve. (Constructed from Fig. 25).
Ordinate: concentration of the anesthetic in units of the outer concentration.
Upper abscissa: distance (r) from the axis in units of the nerve radius (r_0).
Lower abscissa: the area within the distance (r) from the axis in units of the cross sectional area of the nerve.
The cross hatched area indicates the blocked portion of the nerve, the hatched area indicates the fraction of the nerve affected by subminimal concentrations.

Table 20.

Conduction velocity during anesthesia with different xylocaine and procaine base concentrations at times to half action potential amplitude ($t_{0.5}$) and to block ($t_{0.01}$), 22°C.

experiment no.	anesthetic	hydrochloride concentration (C_0) mM	pH	minimum concentration *) (C_m) mM	base concentration (C_B) mM	C_B/C_m	conduction velocity in per cent of velocity in Ringer's	
							$t_{0.5}$	$t_{0.01}$
132	xylocaine	1	6.70	0.09	0.05	0.55	75.8	—
115	—	2	6.88	0.10	0.15	1.5	68.0	35.5
123	—	2	6.90	0.10	0.16	1.6	61.0	34.3
124	—	2	7.00	0.11	0.19	1.7	70.6	37.6
188	—	5	6.72	0.09	0.25	2.8	68.0	35.8
107	—	20	6.50	0.07	0.63	9.0	79.0	42.0
128	—	20	6.99	0.11	1.80	17.2	86.0	50.4
130	—	40	7.09	0.11	4.47	40.6	96.0	47.4
110	—	40	7.12	0.11	4.78	43.5	96.0	50.0
111	—	40	7.16	0.12	5.13	42.8	92.5	50.0
112	—	40	7.44	0.13	8.71	67.3	91.0	52.5
114	—	40	7.49	0.14	9.6	68.6	96.3	46.8
113	—	40	7.52	0.14	10.0	72.4	88.8	56.6
170	procaine	1	7.18	0.022	0.012	0.55	65.0	—
139	—	20	6.00	0.010	0.017	1.7	65.8	34.9
151	—	20	6.20	0.014	0.030	2.1	65.5	32.2
138	—	20	6.50	0.016	0.054	3.4	79.0	37.4
141	—	20	7.80	0.025	1.0	40.8	92.0	43.2
137	—	40	7.80	0.025	2.0	81.6	93.0	46.0

*) minimum concentrations from Fig. 18.

(Fig. 38). The relationship between action potential amplitude and conduction velocity is shown in Fig. 39. With decreasing amplitude (increasing degrees of nerve anesthesia) the conduction velocity decreased.

This progression of events can be explained as the effect of the increasing effect of subminimal concentrations as the anesthetic penetrates into the nerve. The higher the concentration surrounding the active fibers, the greater will be the reduction in conduction velocity. The distribution of concentrations in the nerve shortly after the application of the anesthetic is shown in Figure 40 a: the majority of the active fibers are subjected to

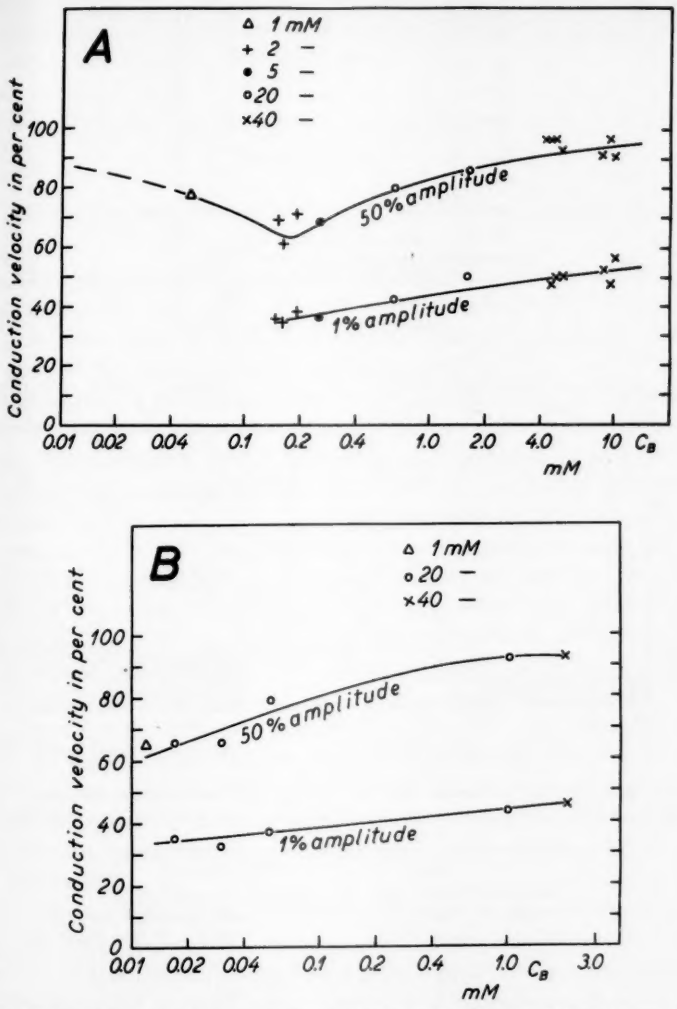


Fig. 41. Correlation between conduction velocity and xylocaine (*A*) and procaine (*B*) base concentration at 50 and 99 per cent block (22°C).
Ordinate: conduction velocity in per cent of the value in Ringer's.
Abscissa: base concentration of the anesthetic in mM (log scale).
The symbols indicate hydrochloride concentrations.

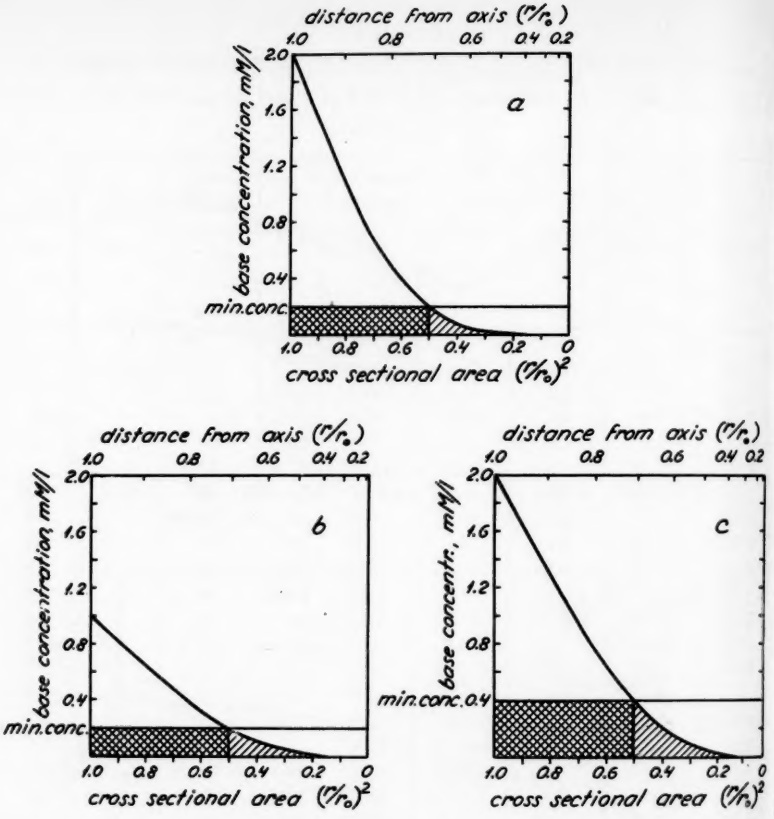


Fig. 42. Diagram to illustrate the effect of subminimal concentrations of the anesthetic on nerve conduction velocity when half of the fibers are blocked. (Constructed from Fig. 25).

outer base concentration in mM	minimum base concentration in mM
a: 2.0	0.2
b: 1.0	0.2
c: 2.0	0.4

Ordinate: base concentration of the anesthetic in mM.
Upper abscissa: distance (r) from axis in units of the nerve radius (r_0).
Lower abscissa: the area within the distance (r) from the axis in units of the cross sectional area of the nerve.

The cross hatched area indicates the blocked portion of the nerve, the hatched area indicates the fraction of the nerve affected by subminimal concentrations.

such low concentrations of the anesthetic that they conduct with normal velocity. A small number of fibers affected by larger but still subminimal concentrations conduct with reduced velocity. Figure 40 b shows the situation when the anesthetic has penetrated further into the nerve, when all active fibers are affected by subminimal concentrations. The overall conduction velocity at this time is therefore less than previously.

The diminution in conduction velocity with different concentrations of the anesthetic.

In 19 experiments the nerves were anesthetized with various concentrations of xylocaine and procaine base (Figs. 41 a, b, Table 20).

At the same degree of anesthesia (50 and 1 per cent amplitude) the conduction velocity diminished with decreasing base concentration for concentrations above the minimum concentration. The effect of a given base concentration was independent of the hydrochloride concentration of the anesthetic. The conduction velocity was lowest at concentrations near the minimum concentration (Chapter 4 page 60). With a concentration less than the minimum concentration (xylocaine base: 0.05 mM) the diminution in conduction velocity was 24 per cent when the action potential amplitude had reached half its initial value as compared with 37 per cent with concentrations just above the minimum concentration. With high concentrations (Fig. 42 a) the portion of the cross sectional area of the nerve affected by subminimal concentrations, which reduce conduction velocity, constitutes a smaller portion of the active area than at low concentrations (Fig. 42 b). This is due to the fact that the concentration gradient in the nerve is greater when the concentration is high. The greatest diminution in conduction velocity is obtained when the active fibers are affected by the highest subminimal concentration. Therefore, for concentrations below the minimum concentration the diminution in conduction velocity is the less the lower the concentration.

Experiments with subminimal concentrations of the local anesthetics when the action potential is reduced in amplitude without being blocked allow to evaluate the correlation between the concentration of the anesthetic and the reduction in conduction velocity. For single nerve fibers TASAKI (1953) has described such a relationship for urethane. Similar findings were made with xylocaine: With 0.05 mM xylocaine base the action potential amplitude levelled off to 27 per cent of its initial value in the course of about one hour. At the same time the conduction velocity levelled off at a value of 56 per cent of the value in Ringer's (18 m/sec, Fig. 38).

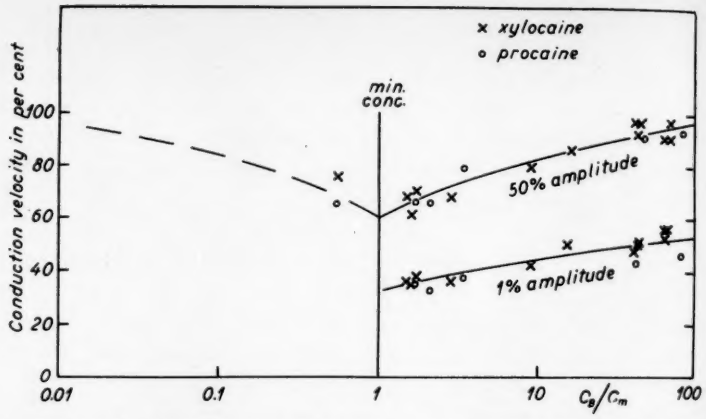


Fig. 43. Correlation between conduction velocity and concentration of xylocaine and procaine base at 50 and 99 per cent block (22° C).
Ordinate: conduction velocity in per cent of the value in Ringer's.
Abscissa: ratio between the base concentration of the anesthetic C_B and the minimum concentration C_m. (Logarithmic scale).

The diminution in conduction velocity with xylocaine and procaine anesthesia.

The diminution in conduction velocity was determined in 13 experiments with xylocaine and in 6 with procaine anesthesia (Table 20).

At the same base concentration the conduction velocity decreased slightly more in the case of xylocaine than in the case of procaine (Figs. 41 a, b). The tendency was clearest when small concentrations were used. Expressing the base concentration in units of the minimum concentration for xylocaine and procaine the conduction velocity was reduced equally for xylocaine and procaine (Figure 43). That is, the minimum concentration of xylocaine and procaine reduced the conduction velocity to the same degree.

The lower conduction velocity for xylocaine than for procaine at the same base concentration can be explained by the difference in minimum concentration and the effect of subminimal concentrations on conduction velocity. For a given outer concentration a higher minimum concentration results in a greater number of active fibers exposed to high subminimal concentrations (Fig. 42 a, c). Hence the conduction velocity is diminished most with the highest minimum concentration. That for the same ratio between base concentration and minimum concentration the diminution in conduction velocity was the same for xylocaine and for procaine can be explained from the theoretical concentration distributions within the nerve (Fig. 25). When

the outer concentration of xylocaine and procaine is the same multiple of the minimum concentration and when the number of blocked fibers is the same (i.e. the same reduction in action potential amplitude) also the same proportion of subminimally affected fibers is present.

Discussion. In the experiments described here it was found that the conduction velocity of the action potential was reduced in that portion of the nerve subjected to a local anesthetic. TOMAN, WOODBURY and WOODBURY (1947) demonstrated that block appeared when the conduction velocity had fallen to half its initial value. TASAKI (1953) found that urethane diminished the conduction velocity in single fibers when the concentration was below the minimum concentration. This alteration in conduction velocity occurred instantaneously after application of the local anesthetic.

Since the conduction velocity returned to normal values in the post-anesthetic section of the nerve its decrease could not be due to a selective block of the most rapidly conducting fibers. The explanation that the conduction velocity is diminished due to the effect of subminimal concentrations on all the fibers is supported by the finding of TASAKI (1953) that urethane in subminimal concentrations diminished the conduction velocity in single fibers. With subminimal concentrations as the cause of the diminution in conduction velocity one can explain: a) that the conduction velocity falls progressively with the progress of anesthesia; b) that it falls most with concentrations of the anesthetic near the minimum concentration and c) that it decreases to the same degree with xylocaine and procaine in concentrations which are the same multiple of the minimum concentrations.

Chapter 9

CLINICAL USE OF LOCAL ANESTHESIA AS RELATED TO THE FINDINGS ON ISOLATED NERVE

Although the isolated nerves investigated were not mammalian and the anesthetic effect was recorded on action potentials from mixed nerves some of the findings reported in this study may bear upon the clinical use of local anesthetics.

In clinical use of local anesthesia, when the anesthetic is applied to the nerve in situ, there are a number of factors influencing the course of anesthesia which are more difficult to quantitate than in isolated nerve: the concentration of the anesthetic depends on the rate of absorption, the degree to which the anesthetic is bound to the tissue surrounding the nerve and, in the case of procaine, the degree of splitting caused by procaine esterase in the tissue. The protolysis of the anesthetic is unknown since it is rare that the pH of the tissue after injection is known. The anesthesia is carried out at a different temperature. Finally, the patients' subjective reports of sensitivity to pain are the indicator for the effect of anesthesia and may be affected by psychic factors.

Susceptibility of different types of nerve fibers to local anesthetics.

In clinical practice local anesthetics are used in the main to block the sensory fibers and particularly the pain fibers. It is therefore of interest to what extent local anesthetics affect these fiber types. Goldscheider (1886) found that sensory receptors vary in their threshold to cocaine in that the sensations of temperature, pain, pressure and touch disappear in that order.

Kochs (1886), Dixon (1905) and Boeminghaus and Kochmann (1929) demonstrated that local anesthetics applied to a mixed nerve block conduction along sensory fibers before the motor fibers are affected (Dixon's law). While motor fibers are predominantly rapidly conducting α fibers in the A group, sensory impulses are conducted by A as well as B and C fibers at various velocities. A pain sensation is experienced first as a rapid, brief,

intense pain conducted by A fibers followed by delayed pain conducted by the slow C fibers. Since the C fibers form the majority GASSER (1943) considers them to be "the principal carriers of pain impulses." The experiments of HEINBECKER and BISHOP (1935), GASSER (1943) and LORENTE DE NÓ (1952) demonstrate that the slow C fibers are blocked before the B fibers which in turn are blocked before the A fibers. According to GASSER (1943) the blocking time is directly proportional with fiber diameter, implying proportionality with conduction velocity. That delayed pain is transmitted by the slowly conducting C fibers explains the fact that the sensation of pain disappears with local anesthesia before other sensory modalities are affected. Since pain also is conducted by more rapid fibers, quick pain would be expected to disappear later than delayed pain.

The suitability of xylocaine and of procaine for clinical local anesthesia.

In the experiments reported here the course of anesthesia was only recorded as it affected the nerve fibers of the A group. To obtain accurate information relevant to the disappearance of pain the minimum concentration of the anesthetic for C fibers should be known. Assuming that the ratio between minimum concentration for A and for C fibers is the same for various local anesthetics, it should be possible to determine from the present experiments whether xylocaine or procaine is best suited for clinical local anesthesia.

Table 21.

Time to half action potential amplitude ($t_{0.5}$) and to block ($t_{0.01}$) with 40 mM xylocaine and 40 mM procaine hydrochloride solutions at pH 7.5.

	base concentration at pH 7.5 (C_B) mM	$t_{0.5}$ sec	$\frac{t_{0.5}\text{ procaine}}{t_{0.5}\text{ xylocaine}}$	$t_{0.01}$ sec	$\frac{t_{0.01}\text{ procaine}}{t_{0.01}\text{ xylocaine}}$
procaine	1.0	72	2.2	394	1.5
xylocaine	10.0	32		263	

mean values of $t_{0.5}$ and $t_{0.01}$ from:
xylocaine experiments no. 160, 112, 114, 113 and 162 (Table 5).
procaine experiments no. 142, 143, 147 and 148 (Table 6).

At the same base concentration (1 mM, p. 81) the blocking time with xylocaine was 40 per cent longer than with procaine. Because of the difference in ionization constants it is, however, necessary to use a 10 times higher concentration of procaine hydrochloride than of xylocaine hydrochloride to obtain the same base concentration at the same pH. Therefore with the same amounts of procaine hydrochloride and of xylocaine hydrochloride at the same pH, xylocaine has a more rapid effect than procaine. For example the time to half and to full block for 40 mM procaine and xylocaine hydrochloride solutions at pH 7.5 (corresponding to 1 mM procaine base and 10 mM xylocaine base) was 2.2–1.5 times less for xylocaine than for procaine anesthesia (Table 21).

Concentration and pH as related to the clinical effect of a local anesthetic.

With the base component as the active anesthetic factor, the time course of anesthesia can be affected by varying the hydrochloride concentration or the pH of the anesthetic solution. From a clinical point of view it is of interest to determine how the variation in these two factors influences the course of anesthesia.

The anesthetic effect of 20 and 40 mM xylocaine hydrochloride at pH 6–7 and 7.35 is shown in Table 22. Doubling the concentration of xylocaine hydrochloride at the same pH results in an 1.2–1.6 times shorter time to

Table 22.

Time to half action potential amplitude ($t_{0.5}$) and to block ($t_{0.01}$) with xylocaine solutions of the same hydrochloride concentration but at different pH.

hydrochloride concentration (C_0) mM	pH	base concentration (C_B) mM	$t_{0.5}$ sec	$t_{0.01}$ sec
20	6	0.21	315	1400
20	7	1.9	78	440
20	7.35	3.8	53	350
40	6	0.4	193	1000
40	7	3.7	54	350
40	7.35	7.5	37	290

$t_{0.5}$ and $t_{0.01}$ are calculated and intrapolated from Fig. 20.

half and full block. A diminution in the pH of the anesthetic solution from 7.35 to 6 resulted in a 3.5–6 times slower fall in action potential amplitude.

To increase the effectiveness of the anesthetic solution it is therefore desirable to use a solution with high pH rather than to increase the concentration of the anesthetic. In clinical practice the solution usually has a pH of 3–5 with the aim of increasing the stability of adrenaline and procaine. It has been assumed that the buffering power of the tissue was sufficient to change the pH of such a solution to about 7.4 almost instantaneously. In support of this assumption TAINTER and MOOSE (1938) found that an acid procaine solution was neutralized by the addition of small amounts of serum. On the other hand BJÖRN (1947) and HOLLER (1953) measured the change in pH in living tissue infiltrated with various local anesthetics and found that the tissue pH remained subnormal for some time. For example after injection of 1 ml of 2 per cent xylocaine and procaine hydrochloride solution at pH 3 the pH of the tissue did not reach normal until after 45 minutes (BJÖRN 1947). Similarly HOLLER (1953) found that after injection of 0.75 ml of 2 per cent procaine hydrochloride at pH 6.23 the pH of the tissue did not return to 7.3 until after 45 minutes.

In a clinical study TAINTER et al. (1938, 1939, 1941) could not demonstrate any difference in the effect of procaine solutions at various pH. Similarly the pH of a xylocaine solution was not of importance for the occurrence of anesthesia, its extent and its duration (HULDT 1953). No information is given, however, as to the pH dependence of the latency of anesthesia and the investigations were carried out solely with high concentrations of the local anesthetics. On the other hand, HARNISCH (1956) found that procaine solutions were clinically more effective at pH about 7.4 than at pH 5.

A number of clinical experiences with local anesthesia may be explained from the effect of pH on the protolysis of the anesthetic. In topical anesthesia of the mucous membranes, the effect is often unsatisfactory. This may be due to the lack of buffer at the tissue surface which can neutralize the anesthetic solution. It has therefore been recommended that a base be added to the anesthetic solution before it is used for surface anesthesia. Local anesthetics have also less effect in infected tissues probably due to their more acid reaction (LETTERER 1959) preventing the anesthetic from being neutralized. The action of a local anesthetic is often unsatisfactory in traumatized tissue (JØRGENSEN 1956). In this case the same change occurs as during infection, i.e. the pH of the tissue is diminished. Therefore local anesthetics are most effective when injected slowly, since rapid injection may traumatize the tissue and cause a diminution in pH.

The surprisingly unsatisfactory anesthesia often associated with the in-

jection of large doses of local anesthetic (JØRGENSEN 1956) may be due in part to traumatization of the tissue and in part to the insufficient buffer capacity of the tissue to neutralize the anesthetic. The same line of reasoning can explain the cases when, after an insufficient nerve block, one places a new depot of the anesthetic around the nerve and still obtains no better anesthesia. LOVESTEDT (1944) recommends in such cases that higher concentrations be used with the second than with the first injection. In view of the fact that the failure is due to the effect of pH it might be more suitable to inject a basic solution instead of using higher concentration.

The addition of adrenaline or other vasoconstrictors is usual in the clinical use of local anesthetics. According to BJÖRN (1947) and HOLLER (1953) this involves a further prolongation of the time required for neutralization of xylocaine and procaine in the tissue possibly because the ischemia diminishes the buffer capacity of the tissue. In addition HILLE and TESKE (1956) believe that adrenaline releases lactic acid. Both explanations imply that the base concentration of the anesthetic is increased only slowly and thereby the latency for the onset of anesthesia is prolonged.

If the anesthetic can remain in contact with the nerve for a sufficiently long time, a small concentration near the minimum concentration is sufficient to obtain full anesthesia even if the time required may be long in that the solution must first be neutralized and the diffusion time into the nerve is long when small concentrations are used. It is of advantage if the pH of the anesthetic solution is high when it is injected: the anesthesia begins more rapidly, an effect is obtained with a smaller concentration and an active concentration is effective for a longer time. That smaller concentrations are sufficient at high pH is of special importance in view of the progressively increasing toxicity with increasing concentration (GOLDBERG 1947).

The effect of the concentration of the anesthetic and of hydrogen ions on the duration of anesthesia.

From the restitution time of the nerve after local anesthesia (Chapter 7) certain conclusions may be drawn as to how the anesthetic influences the duration of anesthesia. To obtain an anesthesia of short duration an anesthetic should be chosen with a high minimum concentration, a high velocity factor and a small thermodynamic ionization constant. The anesthetic solution should be in low concentration and at high pH.

Since the unbound base is the active anesthetic component and the acid component is ineffective one might shorten the duration of anesthesia by transforming the base component to acid in the nerve by lowering the pH

of the nerve. This has however the disadvantage that a lower pH not only reduces the base concentration but also the minimum concentration and thereby the base component at low pH becomes more active anesthetically. The following example demonstrates that this procedure is not practicable:

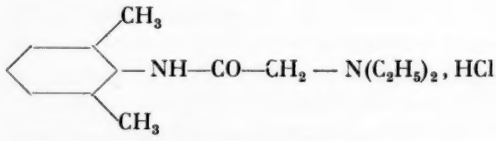
The lowest pH value at which hemolysis does not occur is 5.7 (CHRISTOPHER 1929). A 1 per cent (40 mM) xylocaine hydrochloride solution which contains 6.6 mM xylocaine base at pH 7.3 contains 0.21 mM base at 5.7 pH. This base concentration is however greater than the minimum concentration at pH 5.7, and for this reason the solution still has an anesthetic effect.

APPENDIX 1

CHEMICAL AND PHYSICAL DATA FOR XYLOCAINE AND PROCAINE

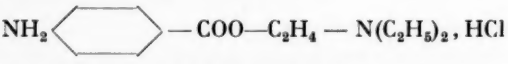
Xylocaine (lidocaine, lignocaine, leostesin). Synthesized by LÖFGREN (1943) who (1948) gives the following data:

structural formula:



molecular weight of the base: 234.3
molecular weight of the hydrochloride: 270.8
thermodynamic ionization constant: ($K_a^{25°}$): $1.40 \times 10^{-8} = 10^{-7.855}$
solubility: "Astra" gives the information that the solubility of xylocaine base in water at 25°C is 0.4 per cent = 17 mM. Since there was used at most 15.5 mM xylocaine base in the experiments reported here the xylocaine base was completely dissolved.

Procaine (novocaine).
Synthesized by EINHORN (1905)
structural formula:



molecular weight of the base: 236.3
molecular weight of the hydrochloride: 272.77
thermodynamic ionization constant: ($K_a^{25°}$): $10^{-8.93}$ (calculated from EISENBRAND and PICHER (1938), see p. 28).
solubility: EISENBRAND and PICHER (1938) found the solubility of procaine base to be 0.13 per cent at 25°C = 5.5 mM. Since the maximal procaine base concentration used in these experiments was 2.04 mM the whole amount was dissolved.

PREPARATION OF RINGER'S AND ANESTHETIC SOLUTIONS

All Ringer's and anesthetic solutions used in the experiments were isotonic (osmotic pressure corresponding to 249 m-equiv) and contained calcium, potassium and glucose in concentrations corresponding to their concentration in normal frog Ringer's as well as 75 mM sodium. The solutions were prepared from 6 stock solutions to obtain concentrations between 0 and 40 mM xylocaine or procaine hydrochloride at pH 5.5 to 7.8.

To prepare the anesthetic solutions 2 per cent xylocaine or procaine hydrochloride solutions were used (stock solution 6).

Xylocaine hydrochloride solution*) is a watery solution to which no disinfectant was added as to the commercial solutions for clinical use. An analysis carried out by "Astra" of the solution used showed that the concentration of xylocaine hydrochloride in the solution was 1.946 per cent.

Procaine hydrochloride solution. Unlike xylocaine which is stable in solution, procaine is hydrolyzed in a watery solution to para-amino-benzoic acid and diethyl-amino-ethanol. The disintegration occurs more rapidly at high pH; the maximal stability is at pH 3.6. Since the splitting is a monomolecular process the time course of the hydrolysis may be described as:

$$x = a(1 - e^{-ct}) \qquad (1)$$

where c is the velocity constant which varies with pH, t is the time in hours, a is the concentration of procaine at the onset and x is that portion which is hydrolyzed after time t. From the velocity constant at various pH levels (TERP 1949) it is seen that procaine is hydrolysed at a rate of 2.3 per cent a day (20°C, pH 7.35). The procaine solution was therefore prepared from crystalline procaine immediately before each experiment.

The osmotic pressure of xylocaine or procaine hydrochloride (denoted BHCl) depends on the degree of protolysis of BH^+ and therefore on pH. In acid solutions BH^+ predominates which, with Cl^-, contributes 2 osmotically active ions. If the solution is rendered alkaline, for example by the addition of NaOH:

$$Na^+ + OH^- + BH^+ + Cl^- = B + Na^+ + Cl^- + H_2O \qquad (2)$$

3 osmotically active particles are formed: B (the uncharged base of the anesthetic), Na^+ and Cl^-. It can therefore be calculated how many m-equiv a

*) The xylocaine solution was kindly made available by "Astra".

certain concentration of anesthetic substance constitutes by multiplying the concentration of hydrochloride by 2 and adding the number of hydroxyl ions which, at the given pH, react with BH^+. The amount of hydroxyl ions is the same as that of the free base formed (see p. 27 and Fig. 3).

To exemplify the preparation of the special Ringer's and of the anesthetic solutions, the constituents of 100 ml of 40 mM xylocaine Ringer's solution are described:

Stock solution 6 contained 2 per cent xylocaine hydrochloride. According to Table 23, 54 ml of this stock solution should be used.

Table 23.

Amount of xylocaine and procaine hydrochloride in the anesthetic solutions.

xylocaine hydrochloride concentration		amount of 2% xylocaine HCl per 100 ml solution	procaine hydrochloride concentration		amount of 2% procaine HCl per 100 ml solution
mM	%	ml	mM	%	ml
1	0.027	1.35	1	0.027	1.37
2	0.054	2.7	2	0.055	2.73
5	0.135	6.75	5	0.136	6.83
20	0.54	27.0	20	0.546	27.3
40	1.08	54.0	40	1.09	54.6

Table 24.

Milliequivalents per liter contributed by a 6.6 mM phosphate buffer at different pH.

pH	mM		m-equiv		
	concentration of Na_2HPO_4 *)	concentration of NaH_2PO_4	Na_2HPO_4	NaH_2PO_4	total
5.5	0.33	6.27	0.99	12.54	13.53
6	0.87	5.73	2.61	11.46	14.07
6.5	2.15	4.45	6.45	8.90	15.35
7	3.95	2.65	11.85	5.30	17.15
7.5	5.52	1.08	16.56	2.16	18.72
7.8	6.00	0.60	18.00	1.20	19.20

*) concentration of Na_2HPO_4 is equal to the concentration of NaOH in Fig. 5.

Stock solution 1. 25 ml of this solution contribute to the anesthetic solution: 2.7 mM KCl (5.4 m-equiv), 1.8 mM $CaCl_2$, $6H_2O$ (5.4 m-equiv), 1.1 mM glucose (1.1 m-equiv) and 6.6 mM NaH_2PO_4, $2H_2O$. Because the phosphate milliequivalents are included in the calculation as phosphate buffer (see Table 24), stock solution 1 contributes 11.9 m-equiv.

In some of the experiments 30 g/l of Dextran were added to the stock solution 1 to add colloid osmotic pressure to the solutions. Since it was found that the addition of Dextran had no influence on the results, this was omitted in the subsequent experiments.

Stock solution 2 contained 0.2 M sodium hydroxide. Sodium hydroxide is added in an amount which, with NaH_2PO_4 from stock solution 1, gives a buffer of the desired pH. Table 24 indicates the concentration of sodium hydroxide (= concentration of Na_2HPO_4) to be added to the solution for this purpose as well as the osmotic pressure the phosphate buffer contributes at various pH levels. In the case of the anesthetic solution there must in addition be added an amount of sodium hydroxide which reacts with the anesthetic acid at the given pH, in other words the same amount which is formed as free anesthetic base (see p. 136). Table 25 shows how much sodium hydroxide should be added to a 40 mM xylocaine Ringer's solution at different pH levels.

Table 25.

NaOH to be added to a 40 mM xylocaine Ringer's with 6.6 mM phosphate buffer at different pH.

pH	NaOH reacting with phosphate buffer*)	NaOH reacting with 40 mM xylocaine HCl**)		total NaOH	ml 0.2 M NaOH per 100 ml solution
	mM	mM	m-equiv	mM	
5.5	0.33	0.13	0.13	0.46	0.23
6	0.87	0.41	0.41	1.28	0.64
6.5	2.15	1.26	1.26	3.41	1.72
7	3.95	3.72	3.72	7.67	3.84
7.5	5.52	9.78	9.78	15.3	7.6
7.8	6.00	15.49	15.49	21.5	10.7

*) values from Fig. 5.

**) according to equation (2) p. 135. The concentration of NaOH = the concentration of free base given in Fig. 3 at various pH.

Stock solution 3 contained 5 per cent $NaHCO_3$. Table 26 shows how much must be added to an anesthetic solution saturated with 3 per cent carbon dioxide to achieve pH 5.5 to 7.8 in the sodium bicarbonate carbon dioxide buffer. Furthermore it may be seen how many milliequivalents per liter the sodium carbonate solution contributed.

Table 26.

Amount of $NaHCO_3$ to be added to an anesthetic or a Ringer's solution (ionic strength 0.134) saturated with 3 per cent carbon dioxide at various pH.

pH	$NaHCO_3$ mM	$NaHCO_3$ m-equiv	ml 5 % $NaHCO_3$ per 100 ml solution
5.5	0.2	0.4	0.04
6	0.7	1.4	0.11
6.5	2.0	4.0	0.35
7	6.6	13.2	1.11
7.5	21.0	42.0	3.53
7.8	42.0	84.0	7.06

Stock solution 4 contained 0.4 M sodium chloride. This was added to supplement the already added sodium from solutions 1, 2 and 3 to raise the sodium concentration of the anesthetic solution to 75 mM. From Table 27 it may be seen how many mM sodium must be added to the anesthetic solution at various pH levels. Table 28 demonstrates how many milliequivalents per liter the sodium chloride solution contributed and how many ml of stock solution 4 must be added to prepare 100 ml xylocaine Ringer's at different pH levels.

Table 27.

Sodium (mM) added to a 40 mM xylocaine Ringer's from the different stock solutions and sodium chloride (mM) to be added to obtain a total concentration of 75 mM sodium.

	pH	5.5	6	6.5	7	7.5	7.8
Table 25	NaOH	0.46	1.28	3.41	7.67	15.2	21.5
stock solution 1	NaH_2PO_4	6.6	6.6	6.6	6.6	6.6	6.6
Table 26	$NaHCO_3$	0.2	0.7	2.0	6.6	21.0	42.0
	NaCl	67.74	66.42	62.99	54.13	32.2	4.9
	total	75.00	75.00	75.00	75.00	75.00	75.00

Table 28.

Amount of sodium chloride to be added to a 40 mM xylocaine Ringer's at various pH.

pH	mM (from Table 27)	m-equiv	ml 0.4 M sodium chloride per 100 ml solution
5.5	67.74	135.5	16.8
6	66.42	133.0	16.6
6.5	62.99	126.0	15.7
7	54.13	108.2	13.5
7.5	32.2	64.4	8.0
7.8	4.9	9.8	1.2

Stock solution 5 contained 0.5 M choline chloride which was added to increase the osmotic pressure to 249 m-equiv. Table 29 indicates how many m-equiv of choline chloride must be added to the xylocaine Ringer's solution at different pH levels. Table 30 shows how many mM choline chloride as well as how much of stock solution 5 must be used to prepare 100 ml xylocaine Ringer's at different pH levels.

Table 29.

Milliequivalents per liter added to 40 mM xylocaine Ringer's from the different stock solutions and milliequivalents choline chloride per liter to be added at various pH to obtain a total osmotic pressure corresponding to 249 m-equiv.

from Table	pH	5.5	6	6.5	7	7.5	7.8
	xylocaine HCl 40 mM	80.0	80.0	80.0	80.0	80.0	80.0
25	Na^+ reacting with xylocaine HCl	0.13	0.41	1.26	3.72	9.78	15.49
	stock solution 1	11.9	11.9	11.9	11.9	11.9	11.9
24	phosphate buffer	13.5	14.1	15.4	17.2	18.7	19.2
26	bicarbonate buffer	0.4	1.4	4.0	13.2	42.0	84.0
28	NaCl	135.5	133.0	126.0	108.2	64.4	9.8
	choline chloride	7.57	8.19	10.44	14.78	22.22	28.61
	total	249.0	249.0	249.0	249.0	249.0	249.0

Table 30.

Choline chloride to be added to 40 mM xylocaine Ringer's at various pH.

pH	m-equiv (from Table 29)	mM	ml 0.5 M choline chloride per 100 ml solution
5.5	7.57	3.8	0.8
6	8.19	4.1	0.8
6.5	10.44	5.2	1.0
7	14.78	7.4	1.5
7.5	22.22	11.1	2.2
7.8	28.61	14.3	2.9

To prepare 100 ml of anesthetic solution at concentrations of 5, 20 or 40 mM xylocaine hydrochloride at every pH level between 5.5 and 7.8 curves have been drawn (Figs. 44 A, B, C) from the values found in Tables 25, 28, 30. Though the tables were intended for the preparation of xylocaine solution, the values could also be used to prepare procaine solutions with a 1 per cent error. As an example Table 31 indicates the components of 100 ml 40 mM of xylocaine Ringer's solution at pH 7.

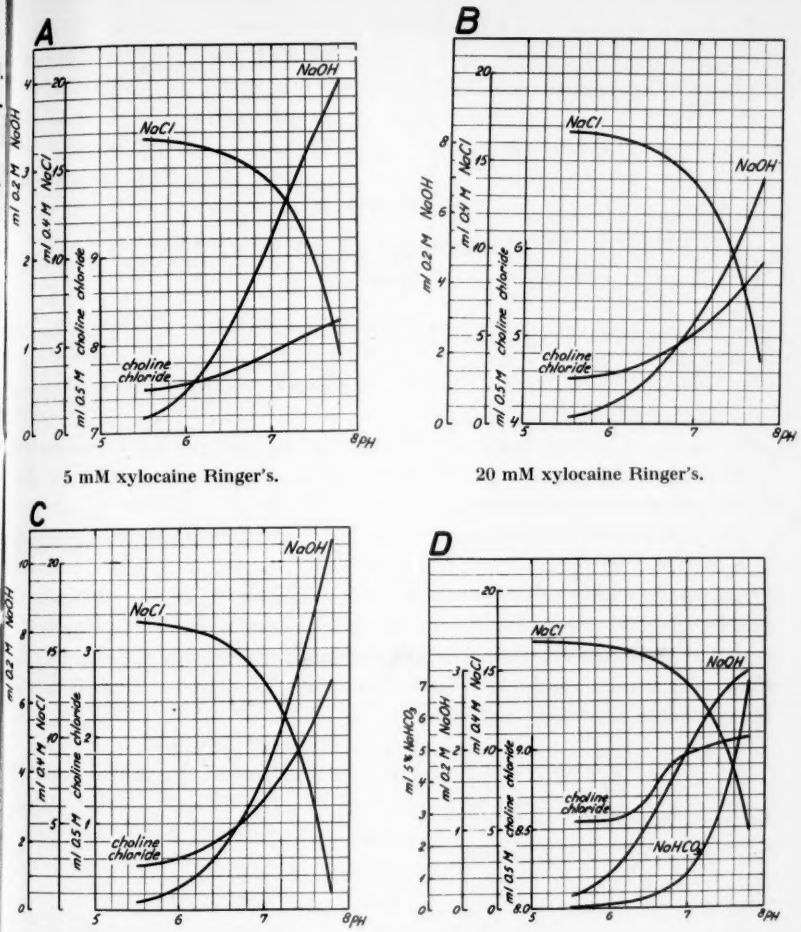
Table 31.

An example of the composition of 100 ml of anesthetic solution at pH 7 made up from 6 stock solutions (40 mM xylocaine Ringer's).

stock solution	content	from	added amount in ml
1	various salts		25.0
2	0.2 M NaOH	Fig. 44 C	3.8
3	5% $NaHCO_3$	Fig. 44 D	1.1
4	0.4 M NaCl	Fig. 44 C	13.2
5	0.5 M choline chloride	Fig. 44 C	1.3
6	2% xylocaine hydrochloride	Table 23	54.0
		total	98.4 ml

+ aqua destillata ad 100 ml

The preparation of the Ringer's solution followed the same principles. 25 ml of stock solution 1 were used for every 100 ml of Ringer's solution. Figure 44 D shows how much must be used of the stock solutions 2, 3, 4 and 5 to obtain solutions at various pH.



5 mM xylocaine Ringer's.

20 mM xylocaine Ringer's.

40 mM xylocaine Ringer's.

Special Ringer's.

Fig. 44. The amount of stock solutions 2, 4 and 5 to obtain 100 ml solution with (*A*) 5, (*B*) 20 and (*C*) 40 mM xylocaine hydrochloride and (*D*) of special Ringer's, a given pH, a sodium concentration of 75 mM and an osmotic pressure corresponding to 249 m-equiv. The amount of stock solution 3 (sodium bicarbonate) for all solutions is shown in (*D*).
Ordinate: ml stock solution.
Abscissa: pH.

APPENDIX 2

DIFFUSION WITH SIMULTANEOUS INACTIVATION

by P. ROSENFALCK

In the mathematical description of the course of anesthesia in whole nerve (Chapter 5) it was not taken into account that there might be a loss of the anesthetic in the nerve on account of chemical reaction. Since procaine undergoes spontaneous non-enzymatic hydrolysis (TERP 1949, SKOU 1954) and possibly hydrolysis in the nerve caused by procaine esterase (NORDQVIST 1952 a) it was necessary to evaluate the influence of a hydrolysis on the theoretical course of anesthesia.

Let us therefore consider an infinitely long cylinder with radius r_0 placed in a large volume containing a substance in concentration C_e which diffuses into the cylinder with an effective diffusion coefficient D. Let us furthermore assume that the substance to a certain extent is metabolised in the cylinder and that the amount metabolised per cm^3 and per second is proportional to the concentration of the substance. The course of diffusion is then determined by the following equation:

$$\frac{\partial C}{\partial t} + s \times C = D\left[\frac{\partial^2 C}{\partial r^2} + \frac{1}{r}\frac{\partial C}{\partial r}\right], \quad \begin{matrix} 0 < r < r_0 \\ t > 0 \end{matrix} \tag{1}$$

with the boundary conditions

$$C = C_e \quad \text{for } r = r_0 \tag{2}$$

$$[C = 0] \quad \text{for } t = 0 \quad \text{and} \quad 0 \leqq r \leqq r_0 \tag{3}$$

In (1) $C = C(r,t)$ represents the concentration of the diffusing substance at distance r from the axis of the cylinder to time t; $s \times C$ represents the amount of the substance metabolized per cm^3 and per second.

The differential equation (1) may be solved by the use of the Laplace-transform. The solution which fulfils the boundary conditions (2) and (3) is:

$$C(r,t) = C_e\left[\frac{I_0\left(r\sqrt{s/D}\right)}{I_0\left(r_0\sqrt{s/D}\right)} - 2\sum_{n=1}^{\infty} e^{-\left(\frac{D\beta_n^2}{r_0^2}+s\right)t} \frac{J_0\left(\beta_n \frac{r}{r_0}\right)}{\left(\beta_n + \frac{sr_0^2}{D\beta_n}\right) J_1(\beta_n)}\right] \tag{4}$$

where J_0 and J_1 are Bessel functions of the first kind while I_0 is a modified Bessel function. β_n are the zeros for J_0.

To evaluate the effect of a breakdown of the substance on the course of diffusion, let us compare expression (4) for the distribution of concentration in the cylinder at various times with the expression (cf. Chapter 5) which disregarded hydrolysis:

$$C(r,t) = C_e \left[1 - 2 \sum_{n=1}^{\infty} e^{-\frac{D\beta_n^2 t}{r_0^2}} \frac{J_0\left(\beta_n \frac{r}{r_0}\right)}{\beta_n J_1(\beta_n)} \right] \tag{5}$$

It is seen from (4) and (5) that the hydrolysis does not influence the course of diffusion significantly as long as the hydrolysis constant s fulfils the following conditions:

$$\frac{I_0(r\sqrt{s/D})}{I_0(r_0\sqrt{s/D})} \approx 1 \tag{6}$$

and

$$s \ll \frac{D\beta^2_n}{r_0^2}, \quad n = 1, 2, 3 \ldots \tag{7}$$

It appears from (4) that condition (6) concerns the final outcome of the diffusion, i.e. the concentrations obtained at time infinity, while conditions (7) are concerned with the time course of diffusion. If these conditions are not fulfilled the course of diffusion with simultaneous chemical reaction diverges from the course of pure diffusion and the concentration within the cylinder never becomes the same as outside it.

The spontaneous hydrolysis of procaine amounts to 0.25 per cent per hour at pH 7.8 (Terp 1949 and Skou 1954). This corresponds to the following value for the hydrolysis constant s in (1):

$$s = 7 \times 10^{-7} \text{ per cm}^3 \text{ and sec.} \tag{8}$$

Let us insert this value in conditions (6) and (7). Since $I_0(r\sqrt{s/D})$ increases as r increases, condition (6) will be fulfilled if

$$\frac{I_0(0)}{I_0(r_0\sqrt{s/D})} \approx 1$$

in other words if

$$I_0(r_0\sqrt{s/D}) \approx 1 \tag{9}$$

since $I_0(0) = 1$.

With $D = 2.1 \times 10^{-7}$ cm²/sec, with $r_0 = 0.4$ mm and with $s = 7 \times 10^{-7}$ $\text{cm}^{-3} \times \text{sec}^{-1}$ it follows that

$$r_0\sqrt{s/D} = 0.07$$

Since I_0 (0.07) = 1.0012 conditions (9) and (6) are fulfilled. This indicates that the splitting of procaine causes no more than a 0.1 per cent lower concentration in the centre of the nerve than outside the nerve when stationary conditions have been reached.

Conditions (7) which concern the time course of anesthesia are with the values of s, D and r_0 given above:

$$7 \times 10^{-7} \ll \frac{1.5 \times 10^{-7} \times \beta_n^2}{16 \times 10^{-4}}$$

or

$$\beta_n^2 \gg 7.5 \times 10^{-3} \qquad (10)$$

Since β_n increases with increasing n, and since $\beta_1 = 2.4$ these conditions are also fulfilled.

Therefore, the spontaneous hydrolysis of procaine is so slow that it does not influence the time course of the diffusion of procaine into the nerve. Even a 10 times greater splitting due to an effect of procaine esterase would not influence the time course of procaine anesthesia.

APPENDIX 3

RESTITUTION OF THE NERVE AFTER ANESTHESIA IN TERMS OF DIFFUSION THEORY

by P. ROSENFALCK

In Chapter 6 it was shown that the progress of anesthesia in whole nerve as evidenced by the gradual diminution of its action potential can be described with good approximation, assuming that the anesthetic diffuses into the nerve with a velocity factor D and that there is a minimum blocking concentration C_m. It was, therefore, of interest to investigate wheter the gradual restitution of the nerve from anesthesia can be described on the same basis.

Considering the nerve as an infinitely long cylinder with radius r_0 the distribution of the anesthetic in the nerve at various times during the progress of anesthesia was given by (Chapter 5):

$$C_A(r, t) = C_e \left[1 - 2 \sum_{n=1}^{\infty} e^{-\frac{D\beta^2_n t}{r_0^2}} \frac{J_0\left(\beta_n \frac{r}{r_0}\right)}{\beta_n J_1(\beta_n)} \right] \tag{1}$$

with C_e representing the outer concentration of the anesthetic and t the time the nerve has been exposed to this concentration.

Let us assume that the anesthesia is continued until the moment when the nerve is fully anesthetized, i.e. when the concentration of the anesthetic at the nerve axis has just reached the minimum concentration C_m. Denoting by $C_A(r,t)$ the concentration of the anesthetic in the nerve during the development of anesthesia this implies that the anesthesia is continued to the time t_0 determined by the following equation:

$$C_A(0,t_0) = C_m \tag{2}$$

If the nerve is thereafter placed in Ringer's solution and if the anesthetic is assumed to diffuse gradually out of the nerve at a rate determined by the velocity factor of the progress of anesthesia then the course of restitution is described by the following differential equation:

$$\frac{\partial C}{\partial t} = D\left[\frac{\partial^2 C}{\partial r^2} + \frac{1}{r}\frac{\partial C}{\partial r}\right], \quad \begin{matrix} 0 < r < r_0 \\ t > t_0 \end{matrix} \tag{3}$$

with the boundary conditions:

$$C(r_0,t) = 0 \text{ for } t > t_0 \tag{4}$$

and

$$C(r,t_0) = C_A(r,t_0). \tag{5}$$

The solution which fulfils these boundary conditions is:

$$C(r,t) = 2C_e \sum_{n=1}^{\infty} \frac{J_0\left(\beta_n \frac{r}{r_0}\right)}{\beta_n J_1(\beta_n)} \left\{ e^{-\frac{D\beta^2_n (t-t_0)}{r_0^2}} - e^{-\frac{D\beta^2_n t}{r_0^2}} \right\} \tag{6}$$

where t_0 according to (1) and (2) is determined by

$$C_m = C_e\left[1 - 2\sum_{n=1}^{\infty} \frac{e^{-\frac{D\beta^2_n t_0}{r_0^2}}}{\beta_n J_1(\beta_n)}\right] \tag{7}$$

From (1) and (6) it is seen that the distribution of concentrations during restitution may be expressed as a difference between two concentration distributions during the development of anesthesia:

$$C(r,t) = C_A(r,t) - C_A(r,t-t_0),\ t \geqq t_0 \tag{8}$$

Utilizing the $C_A(r,t)$ curves given by CARSLAW and JAEGER (1947) (Fig. 25) and equation (8) the time course of restitution was calculated for various concentrations of anesthetic. In Figure 45 A, B and C are shown examples of the distribution of concentrations at various times after the nerve was transferred from the anesthetic solution to Ringer's solution. These distributions illustrate that early in restitution there is diffusion toward the nerve axis simultaneous with diffusion out of the nerve (p. 109).

From the distribution of concentrations the fraction of the cross sectional area of the nerve was calculated in which the concentration of the anesthetic was less than the minimum concentration (cf. p. 86). This fraction was assumed to be a measure of the action potential amplitude. The theoretical restitution courses obtained in this way for various concentrations are compared with the experimental findings in Figure 46. In agreement with the experimental findings, restitution progresses faster with small than with large concentrations of anesthetic. The experimentally measured restitution

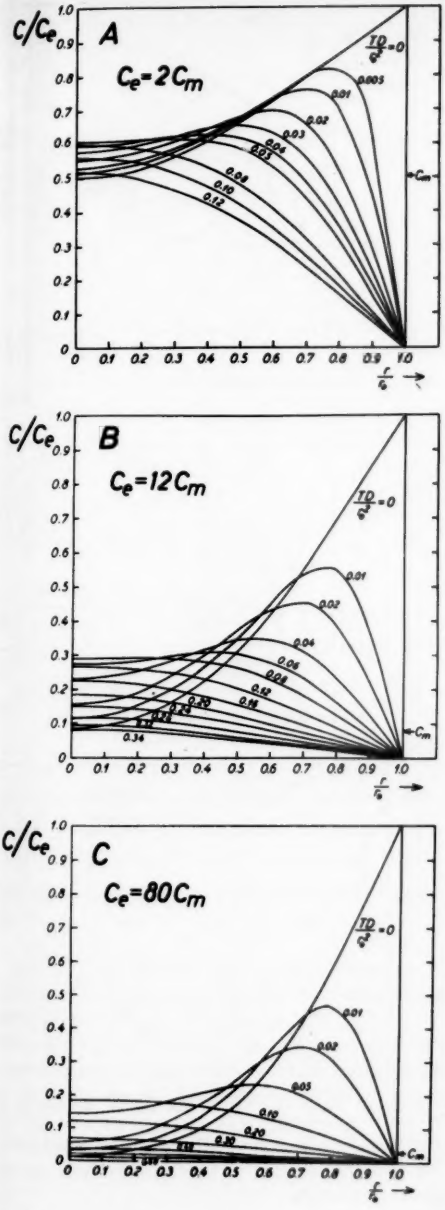


Fig. 45. Calculated distributions within the nerve of concentration of the anesthetic during restitution from anesthesia with different base concentrations.

Ordinate: concentration (C) in units of the outer concentration of the anesthetic (C_e).

Abscissa: distance (r) from the nerve axis in units of the nerve radius (r_0).

The figures on the curves denote the restitution time measured from the time when the anesthetic was substituted by Ringer's given in terms of TD/r_0^2 where T is the time after removal of the anesthetic and D the diffusion coefficient C_m is the minimum concentration.

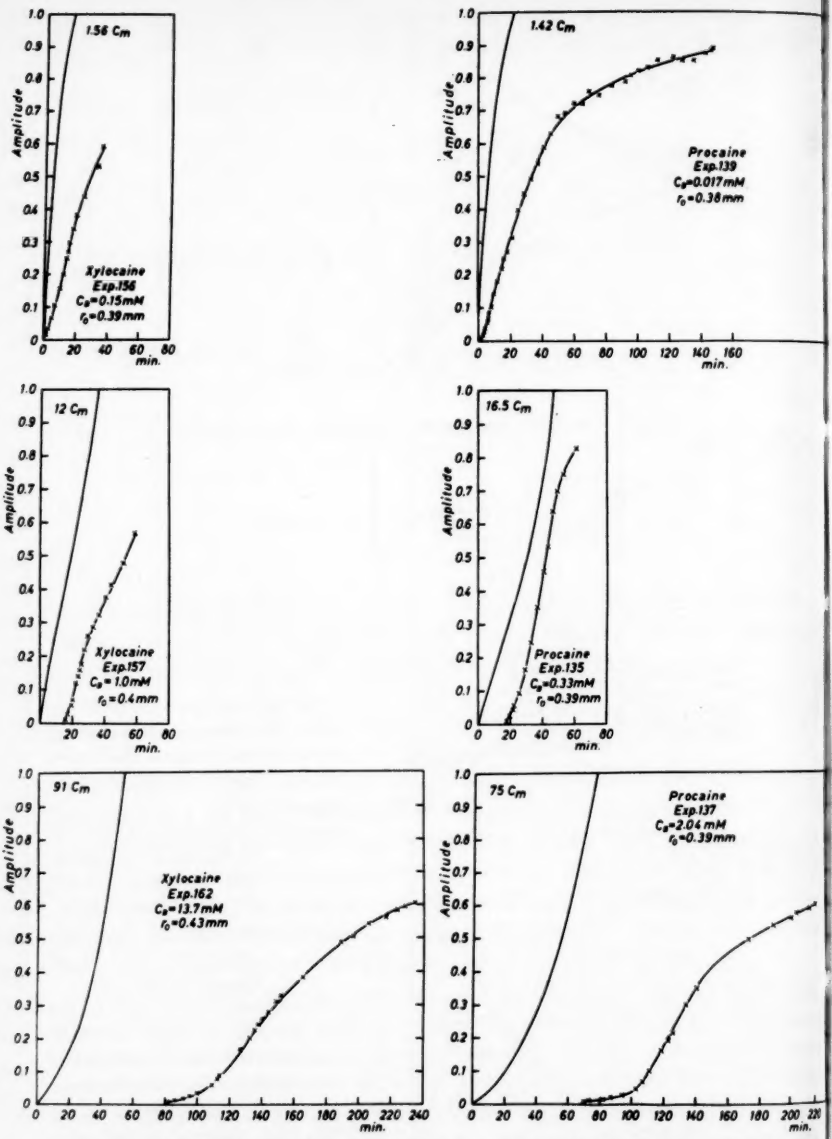


Fig. 46. Experimental and calculated time course of restitution after anesthesia with various concentrations of xylocaine and procaine (given in units of the minimum concentration). The left curve in each diagram is calculated.

Ordinate: action potential amplitude in units of amplitude in Ringer's before anesthesia of the nerve.

Abscissa: minutes from the moment when the anesthetic solution was replaced by Ringer's.

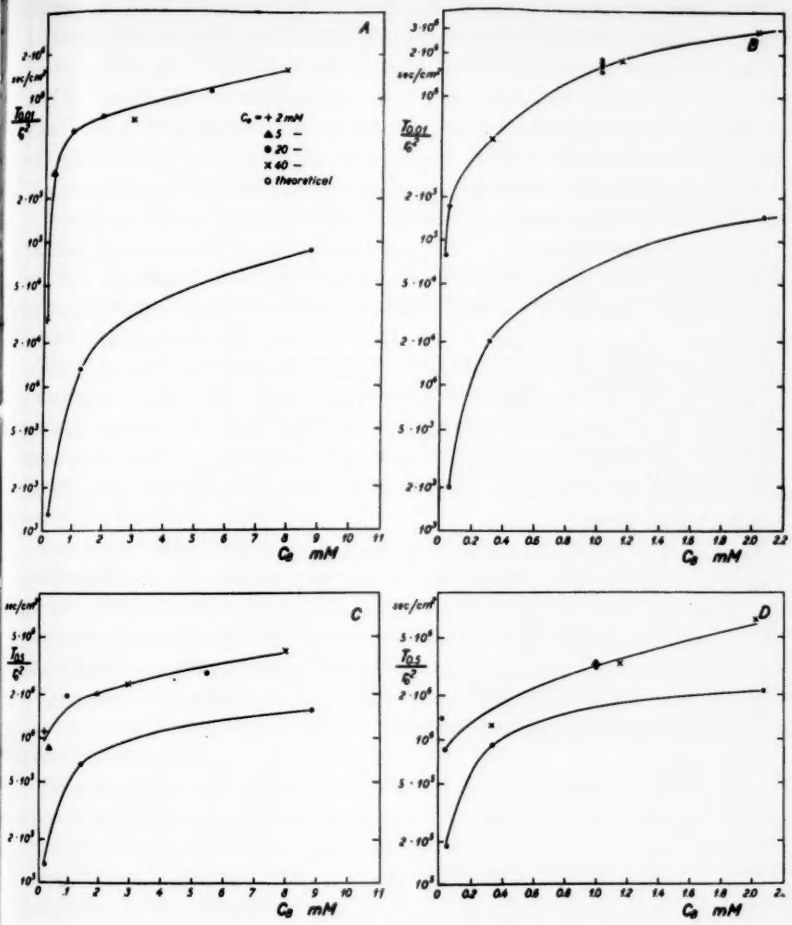


Fig. 47. Experimental and calculated times for 1 ($T_{0.01}$) and 50 ($T_{0.5}$) per cent restitution after anesthesia as a function of the base concentration of the anesthetic. The upper curve in each diagram is experimental.
Ordinate: $T_{0.01}$ and $T_{0.5}$ in terms of T/r_0^2 to compensate for differences in nerve radius (r_0).
Abscissa: base concentration of the anesthetic in mM.
A: $T_{0.01}$ – xylocaine, *B*: $T_{0.01}$ – procaine.
C: $T_{0.5}$ – xylocaine, *D*: $T_{0.5}$ – procaine.
The symbols indicate hydrochloride concentrations (C_0).

lasted, however, considerably longer than to be expected from theory in that the theory does not account for the increasing delay in the onset of restitution with increasing concentration. The times to 1 per cent and 50 per cent restitution were compared with the experimentally found values at various concentrations of xylocaine and procaine (Figure 47 A, B, C and D). The theoretical restitution times were calculated by introducing the experimentally determined minimum concentration and the "velocity factor" (cf. Chapter 6) in equation (8) and the restitution curves derived from it. The experimental times to 1 per cent restitution of the action potential were 20–40 times longer than the theoretical (Figure 47 A and B) and the experimental times to 50 per cent restitution were 2–3 times longer than the theoretical (Figure 47 C and D). In spite of these disagreements between theory and experiment the calculations have given evidence that restitution after anesthesia of a whole nerve is determined to a considerable degree by diffusion of the anesthetic out of the nerve. The cause of the discrepancy between experiment and theory is probably the binding of the local anesthetic to the nerve tissue. Such binding would exert its largest effect at the onset of restitution.

On the other hand, the calculations presented in this section have shown that even with simple diffusion restitution takes much longer than block, the difference being 4 and 12 times respectively with concentrations which are 12 and 80 times the minimum concentration. It is therefore not justified to consider the fact that complete restitution after anesthesia often takes considerably longer than block as an indicator of binding of the anesthetic to nerve tissue (Bennett et al. 1942).

SUMMARY

The time course of local anesthesia (xylocaine and procaine) and the concomitant change in conduction velocity were studied in isolated frog nerve.

The time course of local anesthesia depends on the ratio between the concentration of the active component of the anesthetic and the smallest concentration just sufficient to block the nerve (minimum concentration). The minimum concentration was determined experimentally and it was investigated whether the uncharged base component of the anesthetic was the active portion of the molecule.

The anesthetic penetrates into the nerve by diffusion and it has been investigated to what extent the laws of diffusion can account for the time course of local anesthesia and if it is justified to calculate a minimum concentration and a diffusion coefficient assuming free diffusion of the anesthetic into the nerve (EHRENBERG 1948).

From the time course of the restitution of the nerve impulse after local anesthesia an attempt was made to clarify the factors which determine the course of restitution.

The role of the nerve sheath for the time course of anesthesia was studied by comparing the time course in nerves with and without sheath. To study the effect of subminimal concentrations of the local anesthetics on the nerve fiber membrane the conduction velocity of the nerve impulse was determined during the course of anesthesia.

The relationship of the experimental results to clinical local anesthesia is discussed.

Method.

In most instances the action of a local anesthetic has been investigated by psycho-physical methods or by using the mechanical response of a nerve-muscle preparation as indicator (EHRENBERG 1948, SKOU 1954). These procedures do not allow the time course of anesthesia to be followed with sufficient accuracy. Nor do they permit an evaluation of the influence of the

concentration of the local anesthetic and of the hydrogen ion concentration on the time course.

The change in the amplitude of the nerve action potential has previously been used as indicator of the action of the local anesthetic by BENNETT et al. (1942). These changes are suitable to indicate the time course of anesthesia in nerve with and without sheath as well as the time course of the restitution of the nerve impulse after anesthesia. The action potential amplitude has furthermore been used as a measure for whether the nerve was blocked or not in determining the minimum concentration of the anesthetic. To measure conduction velocity the action potential was recorded from two different points on the nerve at known distance form each other. A difference in arrival time of the action potential during local anesthesia is then an expression of the effect of the anesthetic on the conduction velocity of the nerve impulse.

Sciatic nerves from Hungarian frogs (*Rana esculenta*) were subjected to the anesthetic over a length of 32 mm in a 100 per cent humidified atmosphere of 97 per cent oxygen and 3 per cent carbon dioxide. The nerve was stimulated at its distal end with 0.15 msec rectangular pulses, the intensity being 2–3 times the strength which evoked an action potential of maximum amplitude. In this way the fibers of the A group were stimulated. The action potentials just before and just after the anesthetized portion of the nerve were recorded simultaneously on a double beam oscilloscope. As anesthesia progressed the amplitude of the post-anesthetic action potential diminished, while the pre-anesthetic action potential gave information as to the condition of the nerve during the anesthesia.

By using the action potential amplitude to measure the degree of anesthesia a number of factors must be considered which can affect the amplitude: among these factors the dependence of the amplitude on the variation in distance between the recording electrodes was studied and the influence of the degree of stretch of the nerve was investigated. During the experiments the amplitude can increase due to an increase in the resistance between the recording electrodes as a result of evaporation of the fluid shunt around the nerve. The resistance between the recording electrodes was therefore measured and the amplitude was corrected for any variations.

To investigate which portion of the local anesthetic molecule is the anesthetically active component, the dissociation of the anesthetic was varied by altering the hydrochloride concentration from 1 to 40 mM and by varying the pH of the solution between 5.5 and 7.8 by means of carbon dioxide bicarbonate and phosphate buffer. To ensure the same pH within the nerve as outside it, the nerve was maintained in Ringer's solution at the same pH for one hour before the experiment. All solutions were isotonic and had the

same concentration of calcium, potassium and sodium. This was achieved by reducing the concentration in the Ringer's solution of sodium chloride to 75 mM and by adding the anesthetic or choline chloride until the osmotic pressure corresponded to 249 m-equiv.

The effect of a local anesthetic on action potential amplitude.

When the nerve was subjected to a local anesthetic the action potential amplitude fell instantaneously. As anesthesia progressed the amplitude diminished more slowly. The anesthetic effect of xylocaine and procaine hydrochloride was accelerated with increasing concentration and at increasing pH. Both an increase in hydrochloride concentration and in pH cause an increase in the base concentration of the anesthetic. A fourfold increase in base concentration with unaltered acid concentration reduced the time course of anesthesia by more than 40 per cent. An increase in acid concentration by up to 4.7 times did not affect the time course of anesthesia as long as the base concentration was constant. Thus it seems likely, that the base component of the anesthetic molecule is the anesthetically active factor.

A comparison of the course of anesthesia with xylocaine and procaine showed that anesthesia progressed twice as rapidly with xylocaine as with procaine at the same hydrochloride concentration and pH. This was due to the fact that the dissociation constant of xylocaine hydrochloride was 10 times higher than that of procaine hydrochloride.

In *clinical* use when the most rapid effect and the least toxicity are required one should use a solution with as high a pH as possible rather than a high hydrochloride concentration.

The minimum concentration was determined from the smallest concentration capable of blocking a nerve after at least 6–8 hours exposure. This time of exposure was chosen since the excitability decreases when the isolated nerve remains for 12–24 hours in Ringer's solution (Boeminghaus and Kochmann 1927). Experiments with concentrations only slightly above the minimum concentration of the local anesthetic indicated that an exposure time of 6–8 hours allowed equilibrium. In agreement with Skou (1954) it was found that the minimum concentration for procaine was greater when the pH of the anesthetic solution was increased. The same relationship held in the case of xylocaine base. The minimum concentration for xylocaine base was: 0.04 mM at pH 6.00 and 0.11 mM at pH 7.10. In the case of procaine base the following minimum concentrations were found: 0.013 mM at pH 6.12, 0.021 mM at pH 7.08 and 0.026 mM at pH 7.65. The minimum concentration of procaine was thus considerably lower than that of xylocaine.

The pH dependence of the minimum concentration has been considered to indicate that the base component of the anesthetic molecule is not the sole factor which is active during anesthesia (Skóu 1954). In view of the finding that the time course of anesthesia is the same independent of the hydrochloride concentration (and pH) at which a given base concentration was obtained it is suggested that the pH dependence of the minimum concentration indicates an effect of the hydrogen ion concentration on the membrane itself without invalidating the assumption of the base component as the sole anesthetic factor.

The time course of anesthesia as measured by the diminution in action potential amplitude in relation to the theory of free diffusion.

Ehrenberg (1948) has applied the theory of free diffusion to determine diffusion coefficient and minimum concentration from the blocking time at different concentrations of the local anesthetic. I have investigated whether the time course of the diminution in action potential amplitude at various concentrations of anesthetic substance can be described in terms of free diffusion of the base component into the nerve. If this were the case one would expect:

1) agreement between the experimental and the calculated time course in experiments on nerves with different diameters and using different concentrations of the local anesthetic.

2) that the calculated minimum concentrations be identical with the experimentally determined minimum concentrations, and

3) that the same diffusion coefficient should be determined in experiments with different concentrations of the local anesthetics.

For *xylocaine* a reasonable agreement between theoretical and experimental time course was found until the amplitude was reduced to 15 per cent of its initial value whereafter the experimental time course was slightly slower than the theoretical. On the other hand, the model of free diffusion of the anesthetic substance into the nerve could not be utilized to calculate minimum concentrations in agreement with those determined experimentally. The calculated minimum concentrations increased systematically with increasing outer concentration of the anesthetic. Furthermore, identical diffusion coefficients could not be obtained from experiments at different concentrations. For *procaine* there was less agreement between theoretical and experimental time courses than for xylocaine and the calculated minimum concentrations were not identical with those found experimentally.

That the time course of anesthesia cannot be described solely in terms of

free diffusion must be ascribed to the effect of subminimal concentrations of the anesthetic and to the fact that the nerve represents an inhomogeneous medium. As to the influence of the nerve sheath the relative time course of anesthesia in nerves without sheath is similar to that of intact nerves but diffusion occurred faster. Assuming proportionality between concentration of free and bound anesthetic substance, binding of the anesthetic to the nerve tissue causes an apparent lag in diffusion without affecting the relative time course.

In single nerve fibers a local anesthetic can be characterized by its minimum concentration. In whole nerves, where a time factor in the action of a local anesthetic is involved, the description of the course of anesthesia is facilitated by the introduction of a "velocity factor". This would allow the time course to be predicted for different concentrations and for nerves of different diameter and could be used to compare a local anesthetic under different conditions and different local anesthetics.

Empirically most of the time course of anesthesia could be predicted from the formula for diffusion into a cylinder and the experimentally determined values for minimum concentration. In the case of xylocaine, for concentrations 3–90 times the minimum concentration, agreement was found between experimental and theoretical time courses until the action potential amplitude was reduced to 15–10 per cent of its value in Ringer's. The "velocity factor"*) determined in this way was $3.0 \times 10^{-7} \pm 0.15 \times 10^{-7}$ cm²/sec. In the case of procaine the agreement between experiment and theory was satisfactory only in the range below 35 per cent of the initial action potential amplitude and at concentrations of 3.5–80 times the minimum concentration. The velocity factor in this range was $2.1 \times 10^{-7} \pm 0.1 \times 10^{-7}$ cm²/sec.

Comparison of nerves with and without sheath.

Removal of the sheath resulted in a 2–4 times more rapid anesthesia. As was the case with intact nerves the course of xylocaine anesthesia could be described by diffusion theory when the experimentally found minimum concentrations were used. The "velocity factor" ($8.9 \times 10^{-7} \pm 0.8 \times 10^{-7}$ cm²/sec) was 3 times greater than in nerves with sheath.

With increasing **temperature** the anesthesia progressed more rapidly in that the time to half amplitude and to blocking of the amplitude due to xylocaine was halved when the temperature was increased from 3–13°C. In the range 13–21°C there was no significant change in anesthetic time.

*) "velocity factor" is used instead of diffusion coefficient to emphasize the empirical nature of the mathematical description.

The restitution of the action potential amplitude when the anesthetic was removed after local anesthesia.

The restitution time of the nerve impulse after anesthesia was measured from the moment when the nerve was just blocked and the anesthetic was replaced by Ringer's until the action potential amplitude had regained 1 and 50 per cent of its pre-anesthetic amplitude. Dependent on the base concentration of the local anesthetic the restitution time to 50 per cent amplitude was 2 to 230 times longer than the time to block the nerve to half initial amplitude. The restitution time increased with increasing base concentration of anesthetic. The minimum concentration for xylocaine and procaine base, determined by extrapolating the restitution time to the concentration which gave instantaneous signs of restitution, was of the same order as that determined directly. The restitution time increased to a given limit with the duration of anesthesia. At the same base concentration the restitution time after anesthesia was three times less for xylocaine than for procaine owing to the greater minimum concentration and greater "velocity factor" of xylocaine. At the same hydrochloride concentration and pH the procaine-treated nerve was restored about 1.5 times more rapidly than that exposed to xylocaine. This was due to the fact that xylocaine had the highest base concentration. That the time necessary to restore the action potential after removal of the local anesthetic was longer than the blocking time could be explained in part by the slower diffusion out of than into the nerve and in part by the binding of the anesthetic to the nerve tissue which particularly affects the restitution time.

The conduction velocity of the nerve impulse under the influence of a local anesthetic.

The conduction velocity progressively diminished over that portion of the nerve exposed to a local anesthetic. In the postanesthetic section of the nerve the velocity was unaltered. This demonstrates that conduction velocity was not diminished by selective block of the rapidly conducting fibers. The diminution in conduction velocity must therefore be due to the effect of the anesthetic in subminimal concentrations. This conclusion is supported by the fact that the conduction velocity at the same degree of anesthesia was diminished most by concentrations close to the minimum concentration. In this case, of fibers still able to conduct, a greater number are exposed to high subminimal concentrations than with an external concentration which is large as compared with the minimum concentration. When the outer base concentration was the same multiple of the minimum concentration the reduction in conduction velocity was the same for xylocaine and for procaine.

SAMMENFATNING

(Danish Summary)

Formålet med det foreliggende arbejde har været på isolerede frønerver at undersøge det tidsmæssige forløb af en lokalanæsthesi med xylocain og procain og den samtidige virkning på nervens ledningshastighed.

Anæsthesiens tidsmæssige forløb afhænger af forholdet mellem koncentrationen af anæsthesimidlets aktive komponent og den mindste koncentration, der netop kan blokere nerven (minimumskoncentrationen). Minimumskoncentrationen bestemtes experimentelt, og det undersøgtes, om anæsthesimidlets uladede basedel var den aktive del af molekylet.

Anæsthesimidlet trænger ind i nerven ved diffusion. Man har derfor villet undersøge, i hvor høj grad anæsthesiens tidsforløb kan forklares ud fra diffusionsloven, og om man, som påstået af EHRENBERG (1948), ud fra modelforestillingen om anæsthesimidlets frie diffusion i nerven, kan beregne minimumskoncentrationen og en entydig diffusionskoefficient.

Ud fra det tidsmæssige forløb af nerveimpulsens restitution efter en lokalanæsthesi forsøgte man at afgøre hvilke faktorer, der bestemmer restitutionsforløbet.

Nerveskedens betydning for anæsthesiens tidsforløb undersøgtes ved at sammenligne anæsthesiens tidsforløb i nerver med og uden skede. For at studere virkningen af subminimale koncentrationer af lokalanæsthetika på nervefibermembranen bestemtes nerveimpulsens ledningshastighed under anæsthesiforløbet.

Forsøgsresultaternes betydning for klinisk lokalanæsthesi diskuteres.

Fremgangsmåden.

Virkningen af et lokalanæsthetikum er i de fleste tilfælde undersøgt ved psyko-fysiske metoder eller med muskelkontraktionen fra et nerve-muskel præparat som indikator (EHRENBERG 1948, SKOU 1954). Disse fremgangsmåder tillader ikke at følge anæsthesiens tidsforløb med tilstrækkelig nøjagtighed eller at undersøge indflydelsen af anæsthesimidlets koncentration og brintionkoncentration på tidsforløbet.

Ændringen i nerveaktionspotentialets amplitude er tidligere benyttet som indikator for lokalanæsthetikas virkning af BENNETT et al. (1942). Disse ændringer er egnede til at udtrykke anæsthesiens tidsforløb i nerver med og uden skede såvel som tidsforløbet af nerveimpulsens restitution efter anæsthesi. Aktionspotentialets amplitude anvendes endvidere som mål for, om nerven er blokeret eller ej ved bestemmelse af anæsthesimidlets minimumskoncentration. For at måle ledningshastigheden blev aktionspotentialet afledt fra to forskellige steder på nerven med kendt afstand imellem. En ændring i aktionspotentialets ankomsttid under en lokalanæsthesi kan betragtes som udtryk for anæsthesimidlets virkning på nerveimpulsens ledningshastighed.

Ved forsøgene påvirkes n. ischiadicus fra ungarske frøer (*Rana esculenta*) af anæsthesimidlet på et 32 mm langt stykke. Nerven stimuleres i den distale ende med rektangulære impulser af 0.15 msek's varighed og en stimulationsstyrke 2–3 gange det maximale aktionspotentials tærskel. Herved stimuleres A-gruppens fibre. Aktionspotentialet afledes lige før og lige efter det anæstheserede nervestykke i en fugtighedsmættet atmosfære af 97% O_2 og 3% CO_2 og registreres samtidig på en dobbeltstråleoscillograf. Anæsthesiens fremadskriden fremgår af reduktionen af det postanæsthetisk afledte aktionspotentials amplitude, mens det præanæsthetiske aktionspotential fungerer som kontrol på nervens tilstand under anæsthesien.

Ved at benytte aktionspotentialets amplitude som mål for anæsthesigraden, må der tages hensyn til en række faktorer, der i sig selv kan påvirke amplituden. Forsøg blev således gjort over amplitudens afhængighed af variation i afstanden mellem afledningselektroderne og nervens strækningsgrad. Under forsøgene kan amplituden stige ved forøgelse af modstanden mellem afledningselektroderne på grund af fordampning af væskeshunten omkring nerven. Der foretoges derfor måling af modstanden mellem afledningselektroderne, og amplituden korrigeres for eventuelle variationer.

For at undersøge hvilken del af et lokalanæsthetikummolekyle, der er den anæsthetisk aktive komponent, må anæsthesimidlets dissociation kunne varieres. Dette sker dels ved at ændre hydrokloridkoncentrationen mellem 1 og 40 mM, dels ved variation af opløsningens pH mellem 5.5 og 7.8 ved hjælp af en kuldioxyd-bikarbonatpuffer og en fosfatpuffer. For at sikre, at nervens indre har samme pH som anæsthesimidlet, opbevares nerven i 1 time før forsøget i en specialringeropløsning med den pågældende pH. Alle opløsninger har samme koncentration af Ca^{++}, K^+ og Na^+ og er isotoniske. Dette opnås ved at reducere Ringer-opløsningens koncentration af NaCl til 75 mM, hvorefter der tilsattes anæsthesimiddel eller kolinklorid indtil det osmotiske tryk svarer til 249 m-equiv.

Virkningen af et lokalanæsthetikum på aktionspotentialets amplitude.

Når nerven påvirkes med et lokalanæsthetikum, ses et øjeblikkeligt fald i aktionspotentialets amplitude. I det senere anæsthesiforløb aftager amplituden langsommere. Den anæsthetiske virkning af xylocain- og procainhydroklorid fremskyndes ved stigende koncentration og med forøgelse af pH. Både en forøgelse af hydrokloridkoncentration og af pH betinger en forøgelse af anæsthesimidlets basekoncentration. Forøges basekoncentrationen fire gange med uforandret syrekoncentration forkortes anæsthesiens tidsforløb med mere end 40%. En forøgelse i syrekoncentrationen op til 4.7 gange påvirker ikke anæsthesiens tidsforløb, hvis basekoncentrationen holdes konstant. Det forekommer derfor sandsynligt, at anæsthesimidlets basedel er den anæsthetisk aktive faktor.

En sammenligning af anæsthesiforløbet med xylocain og procain viser, at anæsthesien ved samme hydrokloridkoncentration og pH, forløber dobbelt så hurtigt med xylocain i forhold til procain. Dette skyldes, at xylocainhydrokloridets dissociationskonstant er 10 gange større end procainhydroklorids.

Til **klinisk brug** bør man, hvis man ønsker hurtig virkning og mindst mulig toxicitet, anvende en opløsning med så højt pH som muligt fremfor at øge hydrokloridkoncentration.

Minimumskoncentrationen er bestemt som den mindste koncentration, der kan blokere en nerve efter mindst 6–8 timers påvirkning. Denne påvirkningstid valgtes, fordi irritabiliteten nedsættes, når en isoleret nerve opbevares i Ringer i 12–24 timer (Boeminghaus og Kochmann 1927). Forsøg med koncentrationer, der ligger lidt over minimumskoncentrationen sandsynliggjorde, at en påvirkningstid på 6–8 timer er tilstrækkelig for at opnå diffusionsligevægt. Overensstemmende med Skou (1954) finder man, at minimumskoncentrationen for procain bliver større, når anæsthesiopløsningens pH forøges. Det samme er tilfældet for xylocainbasens vedkommende. Minimumskoncentrationen for xylocainbasen er: 0.04 mM ved pH 6.0 og 0.11 mM ved pH 7.10. For procainbasen finder man minimumskoncentrationerne: 0.013 mM ved pH 6.12, 0.021 mM ved pH 7.08 og 0.026 mM ved pH 7.65. Procains minimumskoncentration er således væsentlig lavere end xylocains. Minimumskoncentrationens pH afhængighed er blevet opfattet som et udtryk for, at anæsthesimidlets basekomponent ikke er den eneste faktor, der er aktiv under anæsthesi (Skou 1954). I betragtning af, at anæsthesiens tidsforløb er det samme, uafhængigt af ved hvilken hydrokloridkoncentration (og pH) en given basekoncentration er opnået, kan man forestille sig, at minimumskoncentrationens pH afhængighed er udtryk for brint-

ionkoncentrationens virkning på selve nervemembranen, uden at det ændrer den antagelse, at basekomponenten er den eneste anæsthetiske faktor.

Det tidsmæssige forløb af anæsthesien målt ved nedgang i aktionspotentialamplituden og teorien for fri diffusion.

EHRENBERG (1948) har anvendt teorien for fri diffusion til bestemmelse af en diffusionskoefficient og en minimumskoncentration ud fra blokeringstiden med forskellige koncentrationer af lokalanæsthesimidlet. I de foreliggende forsøg er det undersøgt, om tidsforløbet af aktionspotentialamplitudens nedgang ved forskellige koncentrationer af anæsthesimidlet kan beskrives ved antagelsen af basekomponentens frie diffusion ind i nerven. Hvis dette er tilfældet, skulle man vente:

1) overensstemmelse mellem det experimentelle og beregnede tidsforløb i forsøg på nerver med forskellig tykkelse og ved anvendelse af forskellige koncentrationer af anæsthesimidlet,

2) at de beregnede minimumskoncentrationer er identiske med de experimentelt bestemte minimumskoncentrationer, og

3) at den samme diffusionskoefficient bestemmes i forskellige forsøg.

For *xylocains* vedkommende finder man rimelig overensstemmelse mellem teoretiske og experimentelle tidsforløb, indtil amplituden er reduceret til 15% af initialværdien, hvorefter de experimentelle tidsforløb er lidt langsommere end de teoretiske. På den anden side kan modelforestillingen om anæsthesimidlets frie diffusion i nerven ikke anvendes til at beregne minimumskoncentrationer, der stemmer overens med de direkte experimentelt bestemte. De beregnede minimumskoncentrationer vokser systematisk med øget ydre koncentration af anæsthesimidlet. Endvidere kan entydige diffusionskoefficienter ikke opnås ved forsøg med forskellige koncentrationer. For *procain* er der mindre overensstemmelse mellem det teoretiske og experimentelle tidsforløb end for xylocain, og den beregnede minimumskoncentration er ikke identisk med den experimentelt fundne.

At anæsthesiens tidsforløb ikke kan beskrives alene udfra formlen for fri diffusion må tilskrives virkningen af subminimale koncentrationer af anæsthesimidlet og den kendsgerning, at nerven udgør et inhomogent medium. Med hensyn til nerveskedens indflydelse svarer anæsthesiens relative tidsforløb i nerver uden skede til det i nerver med skede, men diffusionen foregår hurtigere. Hvis der kan forudsættes proportionalitet mellem koncentrationen af frit og bundet anæsthesimiddel, vil binding af anæsthesimidlet til nervevævet medføre en langsommere diffusion uden at påvirke det relative tidsforløb.

På enkelte nervefibre kan et lokalanæsthetikum karakteriseres ved dets minimumskoncentration. På hele nerver, hvor der indgår en tidsfaktor i virkningen af et lokalanæsthetikum, lettes beskrivelsen af anæsthesiforløbet ved indførelse af en "hastighedsfaktor". Denne skulle tillade at forudsige tidsforløbet for forskellige koncentrationer og for nerver med forskellig diameter og kunne anvendes til at sammenligne et lokalanæsthetikum under forskellige forhold og sammenligne forskellige lokalanæsthetika.

Empirisk kan en stor del af anæsthesiens tidsforløb forudsiges ud fra formlen for fri diffusion i en cylinder i forbindelse med de experimentelt bestemte værdier for minimumskoncentrationerne.

For xylocains vedkommende finder man, for koncentrationer i området 3–90 gange minimumskoncentrationen, overensstemmelse mellem det experimentelle og teoretiske forløb, indtil aktionspotentialamplituden er reduceret til 15–10% af Ringer-værdien. Den tilsvarende "hastighedsfaktor"*) er $3.0 \times 10^{-7} \pm 0.15 \times 10^{-7}$ cm²/sek. For procains vedkommende er overensstemmelsen mellem experiment og teori kun god i amplitudeområdet $<35\%$ i koncentrationsområdet 3.5–80 gange minimumskoncentrationen. "Hastighedsfaktoren" er i dette område $2.1 \times 10^{-7} \pm 0.1 \times 10^{-7}$ cm²/sek.

Sammenligning af nerver med og uden skede.

Fjernelse af skeden medfører en 2–4 gange hurtigere anæsthesi. Som tilfældet er med intakte nerver kan xylocainanæsthesiens tidsforløb beskrives ved hjælp af diffusionsteorien, når de experimentelt fundne minimumskoncentrationer anvendes. "Hastighedsfaktoren" ($8.9 \times 10^{-7} \pm 0.8 \times 10^{-7}$ cm²/sec) var 3 gange større end i nerver med skede.

Med stigende *temperatur* forløber anæsthesien hurtigere, idet tiden til halvering og blokering af amplituden ved påvirkning af xylocain halveres ved en temperaturstigning fra 3–13°C. I området 13–21°C er der ingen signifikant forskel i anæsthesitiderne.

Restitution af aktionspotentialets amplitude, når anæsthesimidlet blev fjernet efter en lokalanæsthesi.

Nerveimpulsens *restitutionstid* efter en anæsthesi måles fra det øjeblik nerven netop er blokeret og anæsthesimidlet udskiftes med Ringer, til aktionspotentialets amplitude igen er 1% og 50% af amplituden i Ringer før forsøget.

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*) "hastighedsfaktor" anvendes i stedet for diffusionskoefficient for at fremhæve, at den matematiske beskrivelse er empirisk.

Afhængig af anæsthesimidlets basekoncentration er restitutionstiden til 50% amplitude 2–230 gange længere, end det tager at anæsthesere nerven til det halve af initialamplituden. Restitutionstiden forøges med voksende basekoncentration. Minimumskoncentrationen for xylocain- og procainbase bestemt ved extrapolation af restitutionstiden til den koncentration, der ville give øjeblikkelige tegn på restitution, er af samme størrelsesorden som de experimentelt fundne. Restitutionstiden forøges indtil en vis grænse med varigheden af anæsthesien. Ved samme basekoncentration er restitutionstiden efter xylocainanæsthesi tre gange mindre end efter procainanæsthesi på grund af xylocains større minimumskoncentration og større "hastighedsfaktor". Ved samme hydrokloridkoncentration og pH restitueres den procainbehandlede nerve ca. 1.5 gange hurtigere end den xylocainbehandlede. Dette skyldes, at basekoncentrationen er størst for xylocains vedkommende. At tiden til restitution af aktionspotentialet efter fjernelse af lokalanæsthesimidlet er længere end blokeringstiden kan forklares dels ved den langsommere diffusion ud af nerven end ind i nerven, dels ved, at bindingen af anæsthesimidlet til nervevævet i særlig grad påvirker restitutionstiderne.

Nerveimpulsens ledningshastighed under indflydelse af et lokalanæsthetikum.

Xylocain og procain nedsætter ledningshastigheden i det nervestykke, der anæstheseres efterhånden som anæsthesien skrider frem. I det postanæsthetiske stykke af nerven er hastigheden imidlertid uforandret. Dette viser, at ledningshastigheden ikke nedsættes ved en selektiv blokering af de hurtigste fibre. Nedsættelsen i ledningshastigheden må derfor skyldes virkning af anæsthesimidlet i ikke blokerende koncentrationer. Denne konklusion støttes af, at ledningshastigheden nedsættes mest af koncentrationer omkring minimumskoncentrationen. I dette tilfælde er der af de fibre, der stadig er i stand til at lede impulser, flere der er påvirket af høje subminimale koncentrationer, end ved en yderkoncentration, der er stor i forhold til minimumskoncentrationen. Når den ydre basekoncentration er samme multiplum af de respektive minimumskoncentrationer, er nedsættelsen i ledningshastighed den samme for xylocain og procain.

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